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BIODEGRADATION OF AIRCRAFT DEICING FLUID COMPONENTS IN SOIL

Baron W. Burke, B.S.
Captain, USAF

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March 1999

Presented to the Faculty of the School of Engineering

of the Air Force Institute of Technology

Air University

In Partial Fulfillment of the

Requirements for the Degree of

Masters of Science in Engineering and Environmental Management

Prof. Daniel E. Reynolds

Dr. Mark N. Goltz, PhD

Dr. Charles A. Bleckmann, PhD

Chairman

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Table of Contents

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Baron Burke

List of Figures

List of Tables

Abstract

Aircraft de-icing fluids (ADFs) are used by commercial and military agencies to ensure safe aircraft operations. Disposal of these spent fluids can pose environmental concerns. Propylene Glycol (PG) is one of the main glycol materials used in ADF, and its biodegradability in various media has been very well documented. However, its high biochemical oxygen demand can pose a severe risk to treatment facilities and water bodies around an airfield. Another unknown is the environmental fate and biodegradability of individual additives in ADFs, such as wetting agents, thickeners, surfactants or corrosion inhibitors like tolyltriazole (TTA).

This research investigates the biodegradation activity of PG alone, TTA alone, and PG with TTA in an aerobic (high-clay) soil environment. This research effort used three test methods to measure the microbial response to these ADF chemical components. Automated respirometry indicated the behavior of the microbial activity through measured oxygen consumption and carbon dioxide production. High performance liquid chromatography (HPLC) was used to measure the residual TTA in soil after respirometry tests were completed. Toxicity tests, such as microbial colony population counts (MCPC) and agar well diffusion tests (AWDT), were used to measure the microbial response to these ADF chemical components.

This research was partitioned into two distinctive phases of investigation. Phase-one analyzed individual and combined ADF chemical components in uncontaminated soil. The presence of TTA, from 25 – 1,000 mg/kg, reduced the maximum respiration rate of 1,000 mg/kg PG alone; however, cumulative respiration over the two-week study

period was nearly the same. Respiration rates in soil exposed to only TTA were not significantly different from background rates.

HPLC analysis was performed after two-weeks of respirometry monitoring in phase-one research. The percentage of recovered TTA ranged from 49 – 56% and 79 – 86%, for 25 and 250 mg/kg TTA alone in soil, respectively. The percentage of recovered TTA ranged from 35 – 44% and 69 – 77%, for 25 and 250 mg/kg TTA with PG (1,000 mg/kg), respectively. The percentage of recovered TTA, with or without PG presence, indicated biodegradation and absorption of TTA within the soil environment. HPLC research was performed by Kellner's (1999) absorption/desorption of measurements of TTA with the same (high-clay) soil.

Toxicity tests were performed on microorganisms/soils from phase-one research. The MPCP indicated no measurable difference between microbial populations of uncontaminated soil versus treated soil with ADF chemical components. AWDT indicated no toxic effects from application of TTA solutions of 5,000 – 10,000 mg/L and PG solutions of 10,000 mg/L, individually and combined, upon microorganism within the test methods.

Phase-two research analyzed the re-application of ADF chemicals on acclimated soils from phase-one research. Specifically, oxygen consumption resulting from reapplication of 1,000 mg/kg PG on acclimated soil (PG 1,000 mg/kg) was compared to one-time application of 1,000 mg/kg PG on the uncontaminated soil. Maximum respiration rates were greater for the acclimated soil compared to the uncontaminated soil.

BIODEGRADATION OF AIRCRAFT DEICING FLUID

COMPONENTS IN SOIL

I. Introduction

1.1 Overview

Glycol based de-icing fluids are used at airport facilities worldwide to prevent snow and ice accumulation on aircraft and airfield surfaces. Glycol based de-icing fluid use ranges from approximately 95,000 L (25,000 gal)/y for a small military base to 5.7 million L (1.5 million gal)/y for a commercial airline [Strong-Gunderson *et al.*, 265]. Typically, a large aircraft will use 3,785 L (1,000 gal) of de-icing fluid [Mericas and Wagoner, 39]. There are two distinctive types of de-icing fluids used on aircraft. Aircraft de-icing fluid (ADF) is primarily used for immediate removal of snow and ice prior to aircraft takeoff. Aircraft de-icing/anti-icing fluid (ADAF) has a longer retention time on aircraft surfaces, thus allowing a longer hold time on the ground prior to takeoff. Both of the aircraft de-icing fluids (ADF and ADAF) have demonstrated their excellent reliability in maintaining safe aircraft operations [Mericas and Wagoner, 39-40]. In this thesis, the term aircraft de-icing fluids (ADFs) will refer to both ADF and ADAF.

The ratio of ADF concentrate to water typically ranges from 50:50 to 10:90 [Safferman *et al.*, 11] before application on the aircraft. This ratio depends on the ADF's manufacturer and weather conditions. ADFs concentrate is mainly glycol with some additives. Extensive studies have shown that glycols are readily degradable under many

environmental conditions. The main environmental concern lies with the high biochemical oxygen demand (BOD) placed upon receiving streams, water bodies, and wastewater treatment plants by the glycols.

Aircraft de-icing fluids also contain other essential additives that serve as corrosion inhibitors, thickeners, and surfactants [Hartwell *et al.*, 1375]. One specific pair of chemical isomers, 5(6)–Methyl–1H–Benzotriazole, are used as additives for corrosion protection [Cancilla *et al.*, 433-434]. Recently, studies by Cornell (1998) and Johnson (1997) investigated the effects on microbial degradation from combinations of tolyltriazole (TTA) and propylene glycol (PG) within a soil environment. The studies were performed in response to proposed "landfarm remediation" of spent ADFs. The results from the investigations were inconclusive. These inconclusive results suggest the need for further investigation. This research will expand our knowledge of tolyltriazole and propylene glycol effects on microbial degradation activity.

1.2 Specific Problem

Aviation operations in cold weather regions require the use of ADFs to keep airfield and aircraft surfaces free from ice and snow. With passenger safety in mind, the Federal Aviation Administration enforces strict requirements for de-icing procedures [Mericas and Wagoner, 39]. After application of ADFs to aircraft or runway surfaces, a significant amount will be deposited upon the airfield. Typically 80% of the fluids are deposited on the ground due to spray drift, jet blast, and wind shearing during taxi and takeoff [Hartwell *et al.*, 1376]. The ADFs typically have two main routes to follow once deposited on the airfield. The ADFs can immediately become part of surface water

runoff, due to the frozen grounds' inability to absorb large amounts of runoff. Diluted ADFs can also be retained in snow pile deposits around the airfield until melting/run-off occurs [Transport Canada, 1985; MacDonald *et al.*, 10-13].

The glycol-based effluents (ADFs and water) eventually migrate into the environment where they might have detrimental effects. Diluted formulations and runoff at 1% deicer solution would have a BOD_5 of around 10,000 mg/L. Untreated raw domestic sewage has a BOD_5 of only 200 mg/L [Sills and Blakeslee, 1992]. The extremely large impact of de-icing fluids on water bodies has prompted pollution controls concerning this effluent. An airport group permit, which requires careful control and disposal with effluents, is issued under the Clean Water Act's Stormwater Regulations, specifically the National Pollutant Discharge Elimination System (NPDES) permit program [Oakley and Forrest, 52; Safferman *et al.*, 11].

The disposal of an ADF effluent can amount to an enormous cost due to the amount of dilution water required to meet treatment plant requirements. Restriction of 1 to 5% glycol concentration is the typical range that the treatment facilities will and can accept [Strong-Gunderson *et al.*, 326]. If glycol is not diluted to these levels, then a "shock load" or very high oxygen demand can occur within a wastewater treatment facility. This shock load can seriously affect the performance of the treatment plant [Metcalf and Eddy, 205].

The costs associated with disposal have prompted some recent investigation into recycling the spent fluids for resale back to manufacturers. In the 1990's, Denver's Stapleton Airport collected glycol solution and effectively sold the effluent when glycol concentrations were above 15% [Backer *et al.*, 58]. Airports considering recycling must

standardize their use of ethylene or propylene glycol because mixed streams of the two compounds have virtually no recycle value [Mericas and Wagoner, 48].

The other option of interest is the investigation for on-site treatment through the application of landfarm bioremedation. Therefore, a fundamental understanding of the interactions between the chemical components of ADFs in soils is crucial before landfarming application could ever become feasible.

1.3 Research Objectives

The purpose of this research was to evaluate the biodegradation of propylene glycol with different levels of tolyltriazole in (high-clay) soil. The mixture and reapplication of these two ADF components were also varied to determine any effects upon soil microorganisms.

Respirometry was used to measure the consumption/uptake of oxygen and the production of carbon dioxide due to the degradation of propylene glycol and tolyltriazole. The microbe rich soil provided an aerobic system for observing the effects on microbial biodegradation from different combinations of the two chemicals. A Micro-Oxymax© "closed circuit" respirometer was used to monitor oxygen consumption and carbon dioxide production.

High performance liquid chromatography (HPLC) was used to analyze the residual amounts of tolyltriazole remaining in the soil once the respirometry experiments were complete. The HPLC data was not a complete representation of all biodegradation, due to chemical and physical process that could not be accounted for. However, it provided supplemental information to compare with the respirometry analysis. The

HPLC analysis also supported Kellner's (1999) thesis on absorption/desorption of tolyltriazole within the same (high-clay) soil.

Microbial colony plate counts (MCPC) and agar well diffusion tests (AWDT) were used to help determine whether the tolyltriazole present in different treatments induced microbial toxicity.

This investigation complements research performed on these two ADF components (Johnson, 1997; Cornell *et al.*, 1997). The respirometry research will address new areas of study, by using a larger variety of tolyltriazole treatments (25 – 1,000 mg/kg) with a fixed propylene glycol (1,000 mg/kg) treatment level, individually and combined in soil. Specific research are listed below:

1. Determine the influence on microbial degradation activity from either propylene glycol or tolyltriazole separately in uncontaminated soil environment.
2. Determine the combined influence on microbial degradation activity of tolyltriazole with propylene glycol in a uncontaminated soil environment.
3. Determine if there is any difference in microbial degradation activity when propylene glycol (1,000 mg/kg) is applied to uncontaminated soil/microorganism and preconditioned soil/microorganisms with propylene glycol.
4. Determine if varied combinations and concentrations of ADF chemical components of tolyltriazole and propylene glycol have a toxic effect upon microbial populations in soil.

1.4 Scope

A phased approach was used to accomplish the scope of this study. The first

phase tested the biodegradability of ADF chemical components (propylene glycol and tolyltriazole) at different concentrations and combinations in previously uncontaminated soil. The second phase of testing compared microbial activity of uncontaminated soil to the activity of ADF acclimated soil/microorganisms. The soils used/monitored in the phase-one studies were used in the phase-two as the acclimated soil/microorganisms.

Control of the test conditions and materials should limit variations in the investigation. Control of experimental conditions included; temperature, light, and moisture within the soil environments. Some constraints and assumptions on the scope of this research are as follows:

1. The same (high-clay) soil was used throughout all experiments.
2. Soil moisture was established at ~60% of field capacity (FC) prior to all respirometer experiments. As the respirometer supplies dry O_2 to the soil, there is a potential for that declining moisture content to reduce microbial metabolism. Long runs (over two weeks) were avoided to reduce this potential influence.
3. All propylene glycol applications on soil were held at 1,000 mg/kg.
4. Adequate nutrients (K, N, P) were present within the soil so as not to limit microbial activity (shown in the independent soil analysis, Appendix A).
5. Adequate aerobic conditions were assumed for all respirometer tests.
6. Uniform preparation techniques were maintained for all experimental runs.
7. Photo-degradation was considered negligible since soil in the respirometry experiments was kept in the dark.
8. Soil and chemicals were maintained in the dark and kept in cool conditions of $4^{\circ}C$ to reduce the potential of chemical degradation between experimental runs.

9. Volatilization of chemicals was assumed negligible. This is assumed based on the chemical characteristics of propylene glycol and tolyltriazole.
10. Adequate numbers of microorganisms were assumed to exist in the soil. As this soil was collected in a natural environment, however it was not tested in any way. The assumptions appear reasonable.
11. Sorption/loss of chemicals to glass equipment used in experiments is assumed negligible. Kellner's (1999) results indicate some absorption of tolyltriazole in the (high-clay) soil. However, minimal loss occurs and is assumed negligible.

1.5 Summary

This research investigated the aerobic microbial biodegradation potential of propylene glycol and tolyltriazole in a (high-clay) soil environment. Microbial respiration is a tool that can measure microbial activity within a soil environment under differing chemical combinations/treatments. HPLC analysis supported respirometry results. MCPC and AWDT are also tools for measuring toxicity effects from various chemical concentrations and mixtures. The results will support a better understanding of the biodegradation effects of two ADF components in a soil.

1.6 Terms Used in this Study

Aerobic – Having molecular oxygen present; growing in the presence of oxygen.

Anaerobic – Living, active, or occurring in the absence of free oxygen.

Aircraft De-icing Fluid (ADF) - Used for the immediate removal of snow and ice from aircraft surfaces.

Aircraft De-icing/Anti-icing Fluid (ADAF) – Used for the immediate removal of snow and ice from aircraft surfaces, along with prevention of snow and ice build up on surfaces for a limited time.

Aircraft De-icing Fluid(s) (ADFs) – Refers to both ADF and ADAF for simplicity in the thesis discussion.

Biochemical Oxygen Demand (BOD) – The amount of molecular oxygen used by microorganisms in wastewater, effluents, and polluted waters for the biochemical degradation of organic material and the oxidation of inorganic material. BOD determination is an empirical test that uses standard laboratory procedures and is conducted over a specified time period, usually five days [Eaton *et al.*, 5-2].

Biodegradation – The microbial process of chemical breakdown of a substance into smaller products caused by microorganisms or their enzymes [Atlas and Bartha, 535].

Hydrophobic Organic Compound – Organic compounds with low solubility in aqueous solutions.

Hydrophilic Organic Compound – Organic compounds with high solubility in aqueous solutions.

Organic – Carbon containing compounds, typically containing carbon-carbon bonds [Brown *et al.*, G-11].

Oxidation – A process in which a substance loses one or more electrons [Brown *et al.*, G-11].

Metabolism – Chemical changes within living cells by which energy is provided for microbial growth and the necessary maintenance of cell life [McKane and Kandel, 9].

Microorganisms – Organisms that exist naturally in the environment such as bacteria, fungi, algae, protozoa, and viruses [Atlas and Bartha, 541].

Micro-Oymax© respirometer – An indirect closed loop respirometer designed to detect extremely low levels of oxygen uptake and carbon dioxide output for a variety of studies involving microorganisms, insects, plants, food, and chemical oxidation [Micro-Oxymax© v6.03, Instruction Manual, 3].

Mineralization – The microbial breakdown of organic materials to inorganic materials brought about mainly by microorganisms [Atlas and Bartha, 541].

Propylene Glycol (PG) – Chemical used in ADF/ADAF; $C_3H_8O_2$, See Figure 1-1 below for structure.

Figure 1-1
Propylene Glycol, 1,2-Propanediol

```
      H    H    H
      |    |    |
 H — C — C — C — H
      |    |    |
     OH   OH   H
```

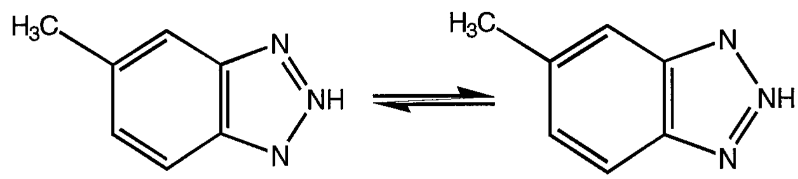
Respirometry – The measurement of the oxygen uptake and the carbon dioxide output associated with biological or chemical systems [Micro-Oxymax© v6.03].

Respirometry Exchange Rate (RER) – The ratio of oxygen uptake to carbon dioxide output, O_2/CO_2 [Micro-Oxymax© v6.03, Instruction Manual].

Statistical hypothesis – claim about the value of a single population characteristic, or about the values of several characteristics [Devore, 304].

Tolyltriazole (TTA) – Chemical used as a corrosion inhibitor in ADF/ADAF, $C_7H_7N_3$. There are two isomers for tolyltriazole. See Figure 1-2 below for structure [Cornell *et al.*, 1997].

Figure 1-2
Tolyltriazole



5-Methyl-1H-Benzotriazole 6-Methyl-1H-Benzotriazole

Field Capacity (FC) – The maximum amount of water that an unsaturated zone of soil can hold against the pull of gravity [Fetter, 639].

Natural Attenuation – The oxidation or breakdown of a substance through natural processes.

Transformation – A reaction that occurs chemically or biologically by means of oxidation or reduction process.

II. Literature Review

2.1 Background on Aircraft De-icing Fluids

Type I ADF is used as a de-icing fluid for aircraft surfaces, while type II ADAF is used as both a de-icing and anti-icing fluid that sticks to aircraft surfaces and inhibits subsequent ice formation during taxi and takeoff [Hartwell *et al.*, 1375]. Although the exact formulations of ADF/ADAFs are proprietary, the main components are glycol materials (90 – 99%) and a small amount of additives (1 – 10%) [SAE, 1992; Cornell, 2; Cancilla *et al.,* 430]. The mixture of concentrated ADF and water can typically be in the range of 50:50 to 10:90 [Safferman *et al.*, 11]. Another difference between ADF/ADAFs are the performance enhancements provided by the additives [Hartwell *et al.*, 1375].

The International Standards Organization (ISO) and Society of Automotive Engineers (SAE), specifically the division of Aircraft Maintenance Chemicals and Materials committee, helps to develop the specifications for commercial ADF/ADAF composition [Boluk and Levesque, 6]. These specifications are guidelines for the fluid application, viscosity, and metal corrosion inhibition qualities for aircraft application. The military specifications covering aircraft de-icing fluids is MIL-A-8243, which specifies two products. First, the military type I ADF, which is propylene glycol based. Second, the military type II ADAF, which is ethylene glycol based (three parts ethylene glycol and one part propylene glycol [Environmental Department of the Naval Facilities Engineering Service Center, 1998].

A directive issued on March 31, 1992 from Brigadier General James E. McCarthy, the Air Force Civil Engineer, placed an immediate USAF-wide prohibition on

the use of ethylene glycol upon all airfield operations. This banning of the ethylene glycol based ADF caused the Air Force to specify propylene glycol based solution to be used throughout all Air Force bases [HQ Air Force Center for Environmental Excellence, 1995].

Type I ADF (commercial) can be a mixture of glycol (ethylene glycol, diethylene glycol, and/or propylene glycol) along with corrosion inhibitors, either 1H-Benzotriazole (BTA) or 5(6)-Methyl-1H-Benzotriazole, common name tolyltriazole (TTA). TTA is used in more ADF formulations than BTA [Cornell, 1997]. The other additives are flame-retardants and surfactants (wetting agents/detergents) made to keep chemicals within the solution. The fluid is typically clear, orange in color [Bausmith, 3; Cancilla *et al.*, 430; Hartwell *et al.*, 1995].

The type II ADAF (commercial) can be a mixture of glycol (ethylene glycol, diethylene glycol, and/or propylene glycol) along with corrosion inhibitors, flame-retardants, and surfactants (wetting agents/detergents), plus thickeners that cause adhesion to the aircraft surface. These thickening agents require a different suite of corrosion inhibitors and surfactants than those used in type I fluids. Typically, the adhesion additive is a polymer, which is neutral and anionic. The fluid is typically clear, pink in color [Bausmith, 3; Cancilla *et al.*, 430; Hartwell *et al.*, 1995].

2.1.1 Environmental Fate of Spent Aircraft De-icing Fluids

Of the ADFs applied, it is estimated that only 16% of the fluid remains on the aircraft surfaces. The amount that falls off the plane is usually collected at the application point using a sump style collection pad. However, the fluids that are retained

do eventually leave the aircraft at some point. An estimated 49% falls on the ground and 35% is lost to wind [Transport Canada, 1988].

The transport of used ADFs that have fallen to the ground is not always direct and simple. ADFs can persist even after the last application of ADFs within a season. An estimated 30% of the de-icier fluid applied will be stored in snow piles to be released during spring rains and snowmelt [Transport Canada, 1988].

2.1.2 Regulations Concerning Spent Aircraft De-icing Fluids

The Environmental Protection Agency (EPA) Storm Water Discharge regulations went into effect on December 17, 1990. These regulations placed storm water under the National Pollution Discharge Elimination System (NPDES) permit program. Under the 1990 regulations, the NPDES permit program now covers effluents previously considered non-point sources [Oakley and Forrest, 1991]. These storm water discharges are associated with industrial activities, including operations such as airports (commercial and military). These industrial activities that result in direct storm water discharge into waters of the United States and storm water discharge through municipal storm sewers are required to obtain NPDES permits from the EPA [Leiter and Funderbunk Jr., 22-23].

The EPA delegated administration of the NPDES program to local state-regulatory agencies. This allowed for some state-to-state difference in handling of the permitting program [Boyd, 1991]. The ultimate outcome was a requirement for proper treatment of stormwater runoff. The water can be treated on site, discharged to publicly owned treatment works, or perhaps recycled [Mericas and Wagoner, 39].

In response to the options available for storm water disposal, new airports began

more active management of these spent ADFs. Newer airports began designing collection and recycling systems, while existing airfields altered their collection and disposal techniques to meet the regulations. This has also led to a renewed interest in handling of these fluids on site.

2.2 Aircraft De-icing Fluids Chemical Components

2.2.1 Properties of Propylene Glycol

The structure of propylene glycol is composed of two OH (alcohol) groups attached to the 1 and 2 carbons (See Figure 1-1). Table 2-1 summarizes the properties of propylene glycol.

Table 2-1
Chemical Characteristics of Propylene Glycol

1,2-Propanediol (Propylene Glycol) Characteristics	Result	Reference
Boiling Point (^oC) at 760 mm Hg	188.2	Sax and Lewis (1998)
Freezing Point (^oC) at 760 mm Hg	-59	Sax and Lewis (1998)
Vapor Pressure (mm HG) at 20^oC	0.08	Sax and Lewis (1998)
Solubility in Water	hydroscopic	Sax and Lewis (1998)
Octanol/Water Partition Coefficient (K_{ow})	$3.89X10^{-2}$	Miller (1979)
Organic Carbon/Water Partition Coefficient (K_{oc})	$2.4X10^{-2}$	Miller (1979)

2.2.2 Properties of Tolyltriazole

The isomers of 5(6)-methyl-1H-benzotriazole, common name "tolyltriazole" (See Figure 1-2), having the methyl group substituted at one of the other positions on the aromatic ring [Cancilla *et al.*, 1996]. The properties of the benzo-ring structure are assumed to make the tolyltriazole compound difficult to degrade. Table 2-2 summarizes

the properties of tolyltriazole.

Table 2-2
Chemical Characteristics of Tolyltriazole

5(6)-Methyl-1H-Benzotriazole (Tolyltriazole) Characteristics	Result	Reference
Boiling Point (°C) at 760 mm Hg	160	PMC Specialties (1996)
Freezing Point (°C) at 760 mm Hg	76-87	PMC Specialties (1996)
Vapor Pressure (mm HG) at 20°C	0.03	PMC Specialties (1996)
Solubility in Water	hydrophobic	PMC Specialties (1996)
Octanol/Water Partition Coefficient (K_{ow})	$3.35X10^{-1}$	Lyman (1982)

2.2.3 Toxicity/Hazards of Propylene Glycol

Literature indicates that pure glycol may be acutely toxic to aquatic life at sufficiently high concentrations. Propylene glycol is not known to be a carcinogen or teratogen [Mallinckrodt, 1997]. The toxicity level of propylene glycol has been established through several studies. Studies reviewed by MacDonald *et al.* (1992) on aquatic organisms (juvenile trout) revealed a median $LC_{50} > 50,000$ mg/L for a 24 hour period [Majewski *et al.*, 1978]. Bridie *et al.* (1979) conducted bioassays on goldfish, which suggested propylene glycol was not acutely toxic at levels below 5,000 mg/L.

Exposure hazards to propylene glycol (pure aqueous) include eye, nose, and throat irritation. High levels become objectionable because of the chemical's odor [Mallinckrodt, 1997].

2.2.4 Toxicity/Hazards of Tolyltriazole

Tolyltriazole is not considered a carcinogen and chronic toxicity data is not available. Research by PMC Specialties Group, indicates a moderate toxicity to aquatic

organisms from the tolyltriazole isomers on Lepomis machorochirus (31 mg/L 96 hr, LC_{50}) and *Daphnia magna* (74 mg/L 48 hr, LC_{50}).

According to the material safety data sheet, tolyltriazole presents moderate risks to health by inhalation, ingestion, or skin absorption [PMC Specialties, 1996]. Thus, appropriate procedures are recommended to prevent opportunities from direct contact with the skin or eyes and to prevent inhalation.

2.3 Biodegradation

The biodegradation process can be influenced by many different conditions. Physical, chemical, and biological conditions directly affect the microorganisms' ability to metabolize a carbon compound into food or energy.

The health and concentration of microbial populations has been directly related to natural or manmade conditions. The competitive environment of nature encourages robust and hardy populations of microbes [Atlas and Bartha, 53]. Other important factors affecting microorganism health and activity are availability of moisture and inorganic nutrients.

Soil microbes require essential mineral nutrients along with a carbon source for unhampered metabolic processes to occur. These essential nutrients for healthy cells are: hydrogen, nitrogen, phosphorus, and sulfur. Hydrogen and oxygen, along with carbon, are essential for synthesis of most organic compounds. Phosphorus is needed for adenosine triphosophate (ATP) and nucleic acids, sulfur for protein, and nitrogen for nucleic acids and protein [McKane and Kandel, 106].

Aerobic metabolism requires oxygen as an electron acceptor for use in the consumption of carbon sources. The pH and temperature of the environmental media can directly influence the health and optimal rate of degradation for microbes.

The benefit of biodegradation is the conversion of contaminants into more environmentally safe compounds, such as carbon dioxide and water.

2.3.1 Effect of Temperature on Biodegradation

Temperature affects microbial degradation of carbon within a soil environment. The activity of aerobic microorganisms indigenous to soil is highest at temperatures of 20 - 30°C [Atlas and Bartha, 218].

Preliminary studies by Klecka *et al.* (1993) indicated that there was an increased biodegradation rate of three different glycol (ethylene, diethylene, and propylene glycol) and five different brands of ADFs, with an increase in soil temperature. The three glycols degradation rates were similar, ranging from 19.7 to 27.0 mg/kg soil per day to 66.3 to 93.3 mg/kg soil per day for samples at 8°C and 25°C, respectively (Klecka *et al.*, 292). This indicated a 3.4 faster rate of microbial degradation for the difference in temperature. Research by Rice *et al.* (1997) indicated a similar relationship between the soil temperature and ethylene glycol mineralization rate.

2.3.2 Effects of pH on Biodegradation

The pH varies in different layers of soil. The upper layer is typically more aerobic and saturated from rainfall than lower layers. The result is that there is more

acidity in the upper layers [Metting, 1993]. Most bacteria and fungi tolerate alkaline pH up to 9.0 but have a pH optima near neutrality [Atlas and Bartha, 234].

2.3.3 Effects of Soil Moisture on Biodegradation

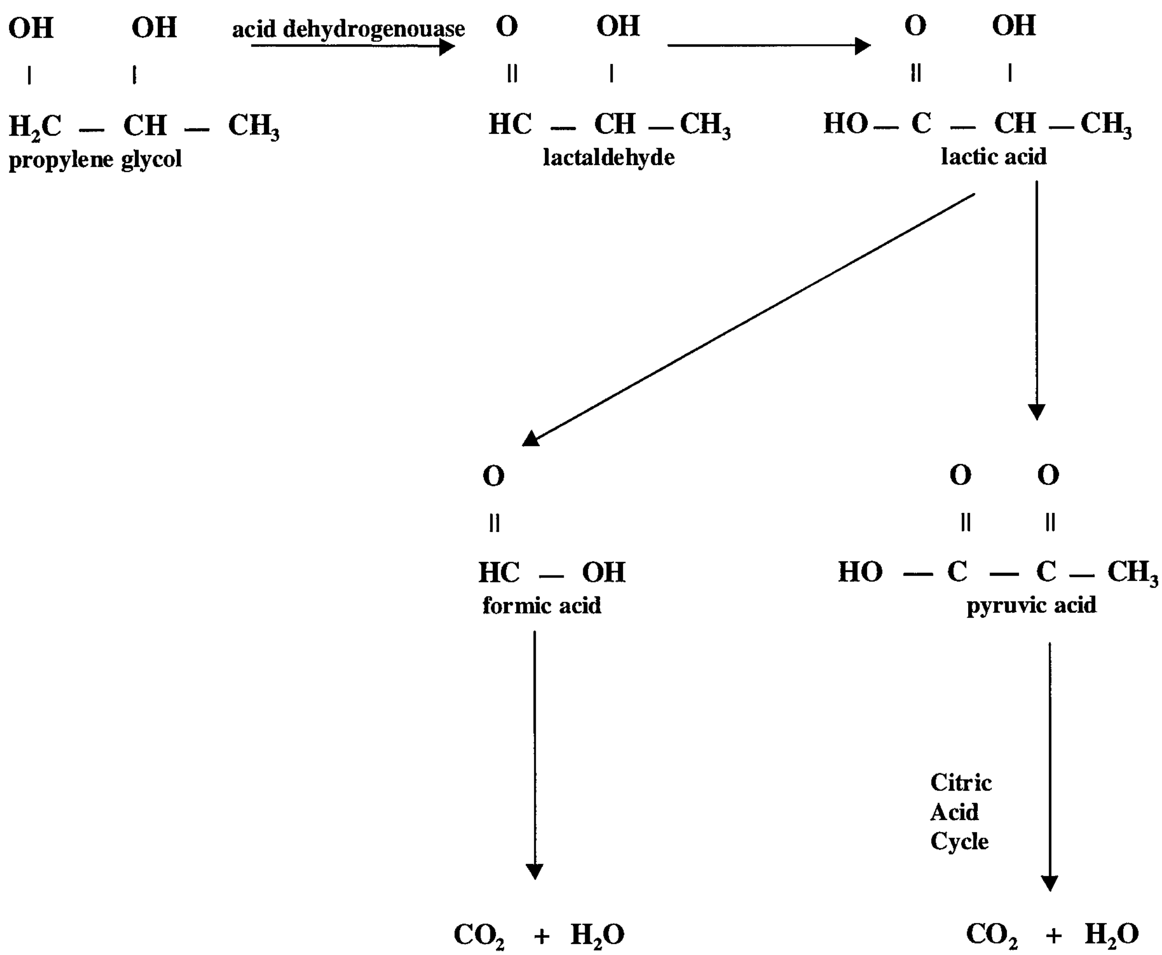
Optimal conditions for activity of aerobic soil microorganisms occurs between 50 and 70% of the water holding capacity of the soil. A higher water content, although not inhibitory by itself, starts to interfere with oxygen availability [Atlas and Bartha, 229].

2.3.4 Biodegradation of Propylene Glycol

Propylene glycol is a low-weight-molecular substance, with a simple structure. The simple structure of propylene glycol permits microorganisms in water and soil environments to readily degrade the chemical in both aerobic and anaerobic conditions. Biodegradation has been demonstrated in water [McGahey and Bouwer, 1992], sewage [Jank *et al.*, 1974; Kaplan *et al.*, 1982; Dwyer and Tiedje, 1983; Raja *et al.*, 1991; Nischke *et al.*, 1996], and soils [Haines and Alexander, 1975; Cox, 1978; Klecka *et al.*, 1993, Kawai *et al.*, 1978; Strong-Gunderson *et al.*, 1995; Buasmith and Neufeil, 1996].

Raja *et al.* (1991) used isolated strains of the bacteria *Pseudomonas* and *Aerobacter* to determine possible pathways of degradation. The *Pseudomonas* degraded the propylene glycol too carboxylic and hydroxycarbonic acids. Further decarboxylation to CO_2 was accomplished by the *Aerobacter* strains [Shupack, 7] as shown in Figure 2-1.

Figure 2-1
Proposed Biodegradation Pathway of Propylene Glycol



Degradation Pathway for Propylene Glycol (Amdur *et al.*, 1991; Raja *et al.*, 1991) as cited from [Shupack, 9]

2.3.5 Theoretical Oxygen Demand of Propylene Glycol

The theoretical oxygen demand (ThOD) for propylene glycol biodegradation may be determined through stoichiometry [Sawyer *et al.*, 528]. The equation in Table 2-3 calculates the amounts (moles) of oxygen to convert an organic carbon material (moles propylene glycol) to carbon dioxide, water, and ammonia.

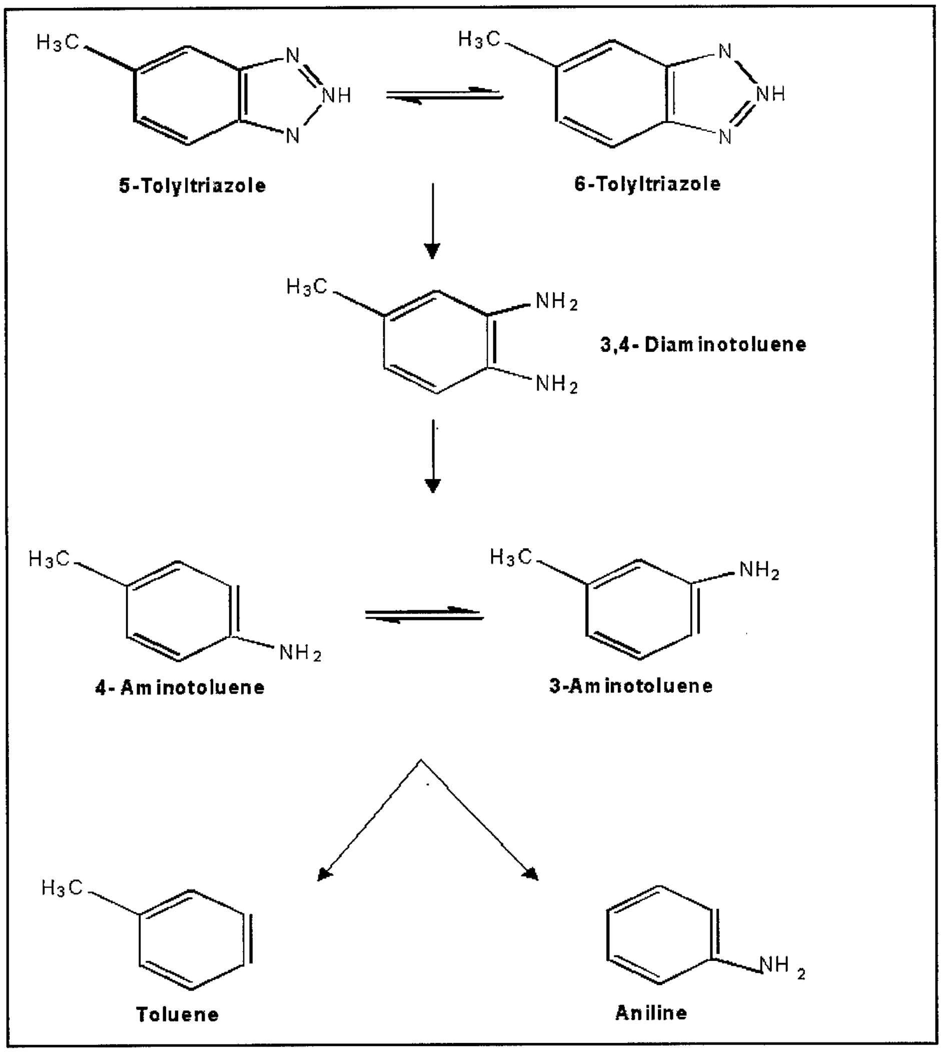
Table 2-3
Calculations for the Theoretical Oxygen Demand of Propylene Glycol

Basic Equation for ThOD:
$C_nH_aO_bN_c + (n{+}a/4{-}b/2{-}3/4c)O_2 \Rightarrow nCO_2 + (a/2{-}3/2c)H_2O + cNH_3$
Propylene Glycol ($C_3H_8O_2$) Stoichiometric Equation:
$C_3H_8O_2 + 4O_2 \Rightarrow 3CO_2 + 4H_2O$
Molar Ratio: O_2: $C_3H_8O_2$ = 4.0
Molar Ratio: O_2:CO_2 = 1.333
Molecular weight $C_3H_8O_2$ = 76.094 mg PG/mole
$\therefore \dfrac{128 \text{ mg } O_2}{76.094 \text{ mg PG}}$
$= 1.68 \text{ mg } O_2/\text{mg PG}$

2.3.6 Biodegradation of Tolyltriazole

The pathway for tolyltriazole biodegradation is still under investigation. It is hypothesized that tolyltriazole degrades anaerobically rather than aerobically [Cornell *et al.*, 1997]. Cornell *et al.* (1997) performed a literature review [Alan R. Katritzky Research Group, 1997; Razo-Flores *et al.*, 1997; Schwarzenbach *et al.*, 1993; Weber, 1994] and proposed the biodegradation pathway shown in Figure 2-2.

Figure 2-2
Proposed Biodegradation Pathway of Tolyltriazole



2.3.7 Theoretical Oxygen Demand of Tolyltriazole

The theoretical oxygen demand (ThOD) for tolyltriazole biodegradation may be determined through stoichiometry [Sawyer *et al.*, 528]. The equation in Table 2-4 calculates the amounts (moles) of oxygen to convert an organic carbon material (moles tolyltriazole) to carbon dioxide, water, and ammonia.

Table 2-4
Calculations for the Theoretical Oxygen Demand of Tolyltriazole

Basic Equation for ThOD:

$$C_nH_aO_bN_c + (n+a/4-b/2-3/4c)O_2 \Rightarrow nCO_2 + (a/2-3/2c)H_2O + cNH_3$$

Tolyltriazole ($C_7H_7N_3$) Stoichiometric Equation:

$$C_7H_7N_3 + 6.5O_2 \Rightarrow 7CO_2 + (-1)H_2O + 3NH_3$$

Molar Ratio: O_2: $C_7H_7N_3$ = 6.5

Molar Ratio: O_2:CO_2 = .9285

Molecular weight $C_7H_7N_3$ = 133 mg TTA/mole

$$\therefore \frac{208 \text{ mg } O_2}{133 \text{ mg TTA}}$$

$$= 1.564 \text{ mg } O_2/\text{mg TTA}$$

III. Methodology

3.1 Overview of Methods Used

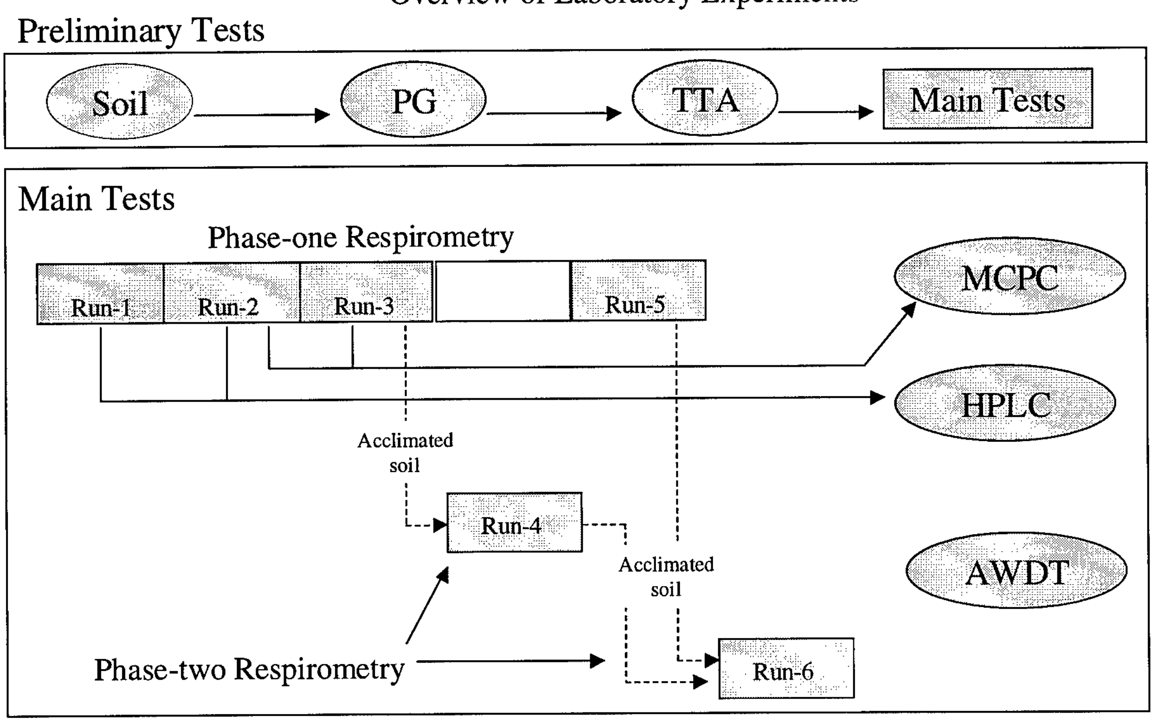
This methodology section describes the materials and procedures used in determining the influence of tolyltriazole on microbial degradation of propylene glycol within a (high-clay) soil. The experiment used in-situ soil microbes to degrade the two ADF chemicals. Microbes activity was monitored by respirometry, which measured oxygen consumption and carbon dioxide production. The specific type of respirometer employed was a Micro-Oxymax© respirometer, built by Columbus Instruments, Inc., Columbus, Ohio. Soils were tested at various concentrations and combinations of tolyltriazole and propylene glycol to understand how these ADF components affect microbial degradation.

Once respirometry tests were complete, two additional analyses were performed on selected spent soils. These analysis were HPLC and toxicity tests. HPLC was used to measure residual tolyltriazole on the spent respirometry soil of phase-one. The first toxicity test was a MCPC, which used spent respirometry soil of phase-one. A water-extract of test soil was added to nutrient agar media. The individual cells grew to colonies and allowed a visual count. The colony totals revealed the population of microbes in soil after interaction with the different ADF chemical concentrations. This allowed a correlation of toxicity effects from the ADF chemicals with the respiration data.

The second toxicity test was an AWDT. This test was a stand-alone test of varying ADF chemical concentrations and combinations. Nutrient agar plates were

allowed to solidify and a microbe rich solution (uncontaminated soil based) was spread on the surface of the nutrient agar. A small well was placed in the center of the agar material and filled with a particular test chemical (propylene glycol, tolyltriazole, or both). The microbes were incubated and colony formations were observed. Suppression of colony formation near the agar well suggested toxicity. An overall layout of all laboratory methods is shown in Figure 3-1. The different chemical treatments for each respirometry run are listed in Appendix E, Table E-1.

Figure 3-1
Overview of Laboratory Experiments



3.2 Laboratory Procedures

3.2.1 Soil Selection

ADF component degradation was analyzed in both a sandy soil and a high-clay soil by Johnson (1997) and O'Malley (1997). Their results showed appreciably more

degradation of propylene glycol in high clay soil rather than sandy soil. This investigation used the same high-clay soil.

3.2.2 Soil Collection

The natural soil in the Dayton, Ohio area is clay based. An open grassy area was selected adjacent to the wooded area that Johnson (1997) and O'Malley (1997) used in their research. A new location was selected in hope that increased microbial population and variety would be found in the grassy area. In many studies, the quantities of microorganisms are significantly less in wooded areas when compared to open grassy areas [Whitman *et al.*, 6578]. In addition, the experiments were designed to model airfield conditions whenever possible.

Soil was collected on September 5, 1998 with sunny-temperate conditions of 31°C and high humidity. The collection was performed with a steel shovel and an 8 liter (2-½ gallon) plastic bucket. Both were pre-cleaned with de-ionized water prior to soil collection. The majority of grass and humic matter was stripped from the collection area within the first 6 cm. The usable soil was collected within the next 20 – 30 cm (vertical layer), in an area of approximately 0.5 square meters. There was no unusual odor or debris encountered during collection. The soil sample was placed in the bucket and covered. The lid was not sealed in order to maintain an aerobic condition. No further soil collections were required, since the 8 liters provided an adequate amount of soil for all of the experimental research.

3.2.3 Soil Preparation

The method described by Klecka *et al.* (1993) was followed. Their method required the soil to be pre-cleaned of large organic matter and sieved through a No. 8. U.S.A. standard testing sieve. A 2 mm square wire mesh was used in place of a No 8. sieve for removal of foreign matter such as leaves, stones, roots, and visible insects.

Experimental runs were conducted over a six-month period. The soil was carefully stored to maintain the quality of soil and microorganisms over this period. The prepared soil was immediately placed in plastic bags (Ziplock™) and refrigerated at $4 \pm 1^{\circ}C$ to slow microbial activity and minimize changes.

3.2.4 Soil Characteristics

The Soil, Water, and Plant Testing Laboratory, Colorado State University, Ft Collins, Colorado, performed an independent analysis of the soil used in the investigation. As indicated in the report (Appendix A), all of the essential nutrients were in ample amounts for support of microbial metabolism. The results from the laboratory are summarized in Table 3-1.

Table 3-1
Chemical Characteristics of the Soil

Organic Matter (%)	Phosphorus (P) (ppm)	Potassium (K) (ppm)	(Mg) (ppm)	Calcium (Ca) (ppm)	pH
2.9	5.3	94.3	2.8	3.0	7.8

The physical characteristics were also analyzed. The results from the independent soil report are summarized in Table 3-2.

Table 3-2
Physical Characteristics of the Soil

% Sand	% Silt	% Clay	ASTM Soil Classification
48	36	16	Loam

3.2.5 Soil Moisture

As discussed earlier, microbial metabolism is directly related to the water content in the soil. Water content tests used by Thomas (1996) were followed to determine the percentage of field capacity (saturated soil moisture). Preliminary tests were performed to determine the optimal water content that would provide adequate mixing/workability of this soil. Soil above 65% field capacity showed clumping and compaction. This was considered unacceptable (potentially anaerobic conditions). The range of 55 – 65% field capacity was established as usable. The final choice of a 60% field capacity was set, and water/solution was added to achieve this level within all the experimental runs.

The reason for beginning all experiments at a relatively high water content arises from the operation of the respirometer. Once the microcosms were closed, no further injections of fluids occurred during an experimental run. Evaporation of water occurred as the respirometer passed dried air over the soil during headspace sampling. Soil moisture tests were performed on the spent soil after respirometry runs. The data revealed an average range of 50 – 55% field capacity after respirometry runs.

3.2.6 Soil pH

The untreated soil had revealed a pH of 7.8 for the soil as reported in the independent soil analysis. No adjustment of pH was done prior to respirometry

experiments due to its near neutral condition. Simple pH tests were conducted before and after the respirometry tests. The data was summarized in Table 3-3.

Table 3-3
Tests on Soil pH used in Respirometry Runs

	Respirometry Test		
Soil Treatment	Before	After	Instrument
De-ionized H_2O	7.8	7.8	HACH
PG_{1000}	7.9	7.8	pH tester
PG_{1000} & TTA_{1000}	7.9	7.8	44450-00

3.2 Treatment Overview

Respirometry experiments were conducted in two phases. Phase-one used uncontaminated soil with varied combinations of ADF chemicals and concentrations. Phase-two used acclimated soil/microorganisms from phase-one tests.

3.2.1 Overview of Treatment Layout for the Respirometer

There are 20 microcosms available within the Micro-Oxymax© respirometer. Phase-one used five microcosms for each treatment type (PG alone, TTA alone, PG & TTA) in experimental runs, along with three microcosms for blank treatments (de-ionized H_2O). Two empty bottles were also used to monitor machine noise and variation. Phase-two used a range of three to five microcosms due to the various treatments and data requirements. Appendix E, Table E-1 contains a detailed layout of all respirometry runs and treatments.

Sampling of high respiration microcosms (propylene glycol in soil) just before sampling low respiration microcosms (blank soil) can be problematic due to carry-over.

The high CO_2 and low O_2 in the sampling ports/sensors/tubing from the first measurement can reduce affect the next microcosm measurement. In an attempt to minimize the effect, an optimal sampling configuration was developed. An example of an optimal bottle layout in shown in Table 3-4.

Table 3-4
Example of Respirometry Treatment Layout: Phase-one, Run-1

Bottle	1	2	3	4	5
Treatment	TTA_{25}	TTA_{25}	TTA_{25}	TTA_{25}	TTA_{25}
Bottle	6	7	8	9	10
Treatment	Empty	Empty	PG_{1000} & TTA_{25}	PG_{1000} & TTA_{25}	PG_{1000} & TTA_{25}
Bottle	11	12	13	14	15
Treatment	PG_{1000} & TTA_{25}	PG_{1000} & TTA_{25}	Soil	Soil	Soil
Bottle	16	17	18	19	20
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}

As the layout demonstrates, treatments of 1,000 mg/kg propylene glycol alone (PG_{1000}), 25 mg/kg tolyltriazole alone (TTA_{25}), and a combination of propylene glycol and tolyltriazole (PG_{1000} & TTA_{25}) are separated by either empty or blank soil microcosms.

3.3.2 Phase-one Treatments

Phase-one used ADF chemicals on uncontaminated (high-clay) soil. The phase-one tests are associated with experimental Run-1, Run-2, Run-3, and Run-5. The choices in ADF chemical concentrations and combinations were developed through preliminary research. Section 3.3.4 provides further explanation on the preliminary research of concentration choices.

3.3.3 Phase-two Treatments

Phase-two respirometry experiments measured the response of acclimated microorganisms from phase-one soil. Propylene glycol at 1,000 mg/kg was the only ADF chemical and concentration that was reapplied. Set-up and choice of phase-two treatments were developed from results of phase-one respirometry data. The phase-two tests are associated with experimental Run-4 and Run-6.

3.3.4 Microcosm Preparation for Respirometer Analysis

As stated earlier, the workable field capacity was established at ~60% from preliminary tests. Previous experiments by Johnson (1997) and O'Malley (1997) have shown that during periods of rapid respiration the O_2 levels fell below the respirometers lower-detection limit (19.29% O_2). The O_2 depletion was due to large soil amounts (thus many microbes) and high concentrations of propylene glycol (food source).

Shortening the sampling interval and lengthening the duration of refreshing O_2 was considered. However, the respirometer cycle time was already near six hours for the 20 microcosms. Microbial respiration rate was the only other parameter to adjust.

The preliminary tests showed a soil mass of 50 gm along with a propylene glycol concentration of 1,000 mg PG/1 kg soil would be optimal. The 50 grams at 60% field capacity soil would consist of 45 grams of uncontaminated soil (semi-dry) with 5 mL (5 gm) of solution. Calculations are provided in Appendix B.

Tolyltriazole solubility in water and water-propylene glycol solutions were tested to determine their interaction. The interaction being tested was the ability for tolyltriazole to dissolve equally in both base liquids. A consistent solution (no

granules/flocculent) of tolyltriazole was desired in the solution for accuracy in the treatment dose of soil. The interactions were measured through range finding tests of concentrations and temperatures, summarized in Table 3-5.

Table 3-5
Tolyltriazole Saturation Points in Aqueous Solution

Concentration of TTA	5,000 mg/L	5,250 mg/L	5,500 mg/L	5,750 mg/L	6,000 mg/L	6,250 mg/L	
TTA in 10,000 mg/L PG solution	No Floc	Floc	Floc	Floc	Floc	Floc	4°C
TTA in de-ionized H_20 Only	Floc	Floc	Floc	Floc	Floc	Floc	
TTA in 10,000 mg/L PG solution	No Floc	No Floc	No Floc	No Floc	Floc	Floc	25°C
TTA in de-ionized H_20 Only	No Floc	No Floc	Floc	Floc	Floc	Floc	

Concentration of TTA	7,500 mg/L	8,000 mg/L	8,500 mg/L	9,000 mg/L	9,500 mg/L	10,000 mg/L	
TTA in 10,000 mg/L PG solution	No Floc	No Floc	No Floc	No Floc	No Floc	No Floc	43°C
TTA in de-ionized H_20 Only	No Floc	No Floc	Floc	Floc	Floc	Floc	

Note: After 43°C heat is removed, TTA precipitates out of both solutions

Floc = initial flocculent (floc) of TTA granules in solution
Floc = heavy flocculent (floc) of TTA granules in solution

Table 3-5 reveals that tolyltriazole did not flocculate in water or water-propylene glycol up to the 5,000 mg/L at 25°C. The application of heat allowed higher concentrations of tolyltriazole to dissolve in the solutions, which allowed consistent solution concentrations for application on soil.

To prevent chemical and microbial degradation of the solutions between experimental runs, a protocol of generating fresh batches of solution was adopted. The calculations of mass and volumes for preparing the concentrations of ADF solutions are found in Appendix C.

The propylene glycol solution (10,000 mg/L) was prepared from a reagent grade (Mallinckrodt OR, 1925; 1,2-Propanediol) chemical to ensure purity and concentration. Five grams of propylene glycol was diluted into 500 mL of de-ionized water in a volumetric flask (Pyrex®), with a ground glass stopper. It was mixed with a magnetic

stirrer (Corning™, PC -210) for approximately one hour, at room temperature (~22°C) in lighted conditions.

The tolyltriazole only solutions (250 – 7,500 mg/L) were prepared from commercial grade COBRATEC TT-100 (sample 4239701). Solid phase pellets of the tolyltriazole were ground into powder in a pre-cleaned crucible. The appropriate amounts of the powder were mixed with 200 mL of de-ionized water in a volumetric flask, then mixed on a heated/electro-magnetic stirrer (PMC™, 525A).

- Concentrations of 250 – 5,000 mg/L were maintained at ~22°C (room temperature) and stirred for eight hours in unlighted conditions.
- Concentrations of 5,000 – 10,000 mg/L were heated to 43°C for 15 minutes, then stirred for eight hours in unlighted conditions at ~22°C, then reheated to 43°C for 15 minutes prior to application on the soil.

The combined solution of propylene glycol with tolyltriazole was then prepared with the same chemicals. The selected amount of tolyltriazole was added to 200 mL of propylene glycol solution (10,000 mg/L) and mixed in a volumetric flask with a ground glass stopper. The chemicals were mixed upon an electro-magnetic stirrer for approximately eight hours, at the appropriate temperature, as related to the tolyltriazole concentration in unlighted conditions.

The soil was allowed to adjust to room temperature (~22°C) in advance of mixing with solutions. The acclimatized soil required less time to equilibrate at the respirometers incubator temperature (25°C).

The respirometers microcosm bottles (250 mL, Pyrex) were pre-cleaned with de-ionized water. The soil and 5 mL of test solution (de-ionized water, propylene glycol, tolyltriazole, or propylene glycol with tolyltriazole) was added to the bottle and stirred.

A stainless steel spatula was used to mix the contents for five minutes per microcosm. This ensured all soil was fully mixed and wetted. The spatula was cleaned with de-ionized water before mixing other microcosm/treatments.

3.4 Respirometer

3.4.1 Overview of Respirometer System

The respirometer used a "closed loop" system configuration for measuring O_2 consumption and CO_2 production gases from each individual microcosm. Details on use of the Micro-Oxymax© respirometer may be found in Totten (1995), Baker (1995), and Thomas (1996).

3.4.2 Respirometer Calibration

Prior to each experimental run, several calibration adjustments were performed to ensure accurate O_2 and CO_2 measurements. The CO_2 sensor was zeroed through the introduction of 99.999% pure nitrogen (PRAXAIR Company, certified mixture). The nitrogen-only atmosphere ensured a zero reference point for calibration. Then a laboratory grade (Liquid Carbonic Company) mixture of CO_2 (0.501%) and O_2 (20.4%) was introduced. The CO_2 and O_2 sensors were adjusted to match the standard gas and then set/locked for the remainder of the experimental run. Each new experimental run required re-calibration prior to initiating the respirometers automated sampling program.

Leak checks of each microcosm (250 mL bottle and tubing) were performed by the machine through a self-diagnostic program that verifies "pass or fail" of all the systems. The "passing" range of $\pm$ 0.2 mL/min leakage is allowed for one out of three

times tested on each of the 250 mL bottles tested [Micro-Oxymax© Software manual, 19].

In response to a recommendation from Johnson (1997) and O'Malley (1997), the respirometer was relocated to a climate-controlled laboratory. The purpose was to reduce atmospheric humidity and temperature variations that seemed to cause erratic calibration checkouts. In addition, the oxygen sensor was replaced on July 19, 1998, since O_2 sensitivity began to rise above specified limits. The machine was then inspected and calibrated at Columbus Instruments on August 21, 1998 to ensure the machine met factory tolerances.

3.4.3 Respirometer Parameter Controls

The experimental runs were conducted during a two-week period using controlled environmental parameters. Temperature was maintained in an incubator (Lab Line™, AMBI-HI-LO) at $25 \pm 1^{\circ}C$. Photo-degradation was eliminated throughout all experimental runs, since the incubator shielded the microcosms from light. The refreshed air provided to the respirometer was passed through a two-stage moisture absorbent system. First, through a stand-alone absorbent system (DRIERITE™, $CaSO_4$) then through a desiccant, containing magnesium perchlorate (GFS chemical, $Mg(ClO_4)_2$). Low moisture air was required for accurate measurements of CO_2.

3.4.4 Data Collection and Conversion

The experimental software (Micro-Oxymax© V6.03) for the respirometer provided detailed information/data for automated sampling. Every six hours a sample point was captured for each of the 20 microcosm bottles for the entire two-week range of

the experimental run. Table 3-6 summarizes the respirometers measurements.

Table 3-6
Respirometer Output Data

	Used for Data Analysis				Used to ensure machine is functioning properly and within ranges desired		
Title	O_2 Consumption	CO_2 Production	Rate of O_2 Consumption	Rate of CO_2 Production	Temperature	% O2	% CO2
Units	(uL)	(uL)	(uL/hr)	(uL/hr)	^oC		
Precision	0.1	0.1	0.1	0.1	0.01	OK if >19.29%	All Ranges

Note: If O_2% falls below 19.29%, the machine cannot account for the actual O_2 volume for the sample interval.

3.5 High Performance Liquid Chromatography (HPLC)

3.5.1 Overview of HPLC Detection of Tolyltriazole

HPLC analysis was performed on a Hewlett Packard 2170 HPLC using ultraviolet detection. The HPLC used a Hewlett Packard auto-sampler in conjunction with software support of Hewlett Packard Chem-Station® for liquid chromatography systems (Rev. A. 4.02). The HPLC analysis was used to measure the residual tolyltriazole absorbed on the soil after respirometry. HPLC tests were also performed on freshly inoculated soil, which was immediately processed/extracted in an attempt to measure dissolved phase of tolyltriazole in the soil. The analysis of residual amounts of tolyltriazole before and after respirometry tests aided in identifying as many degradation pathways (physical, chemical, and biotic) as possible.

3.5.2 Extraction Method for Tolyltriazole in Soil

Approximately 12 – 13 gm of soil was placed in a 40 mL bottle (Fisher Brand, EPA vials). 15 mL of methanol (Fisher Chemicals, HPLC Grade) was then added to the

soil for extracting the remaining tolyltriazole. The 40 mL bottles were mixed on a rotator (Glas-Col, Laboratory Rotator) for 24 hours and then centrifuged (International Clinical, Model 4182C) for 20 minutes at a speed of 1,000 rpm. Upon completion, the liquid phase of the sample was carefully extracted and filtered (Gelman Sciences Acrodisc, 0.2 μm filter). The sample was then ready for HPLC analysis.

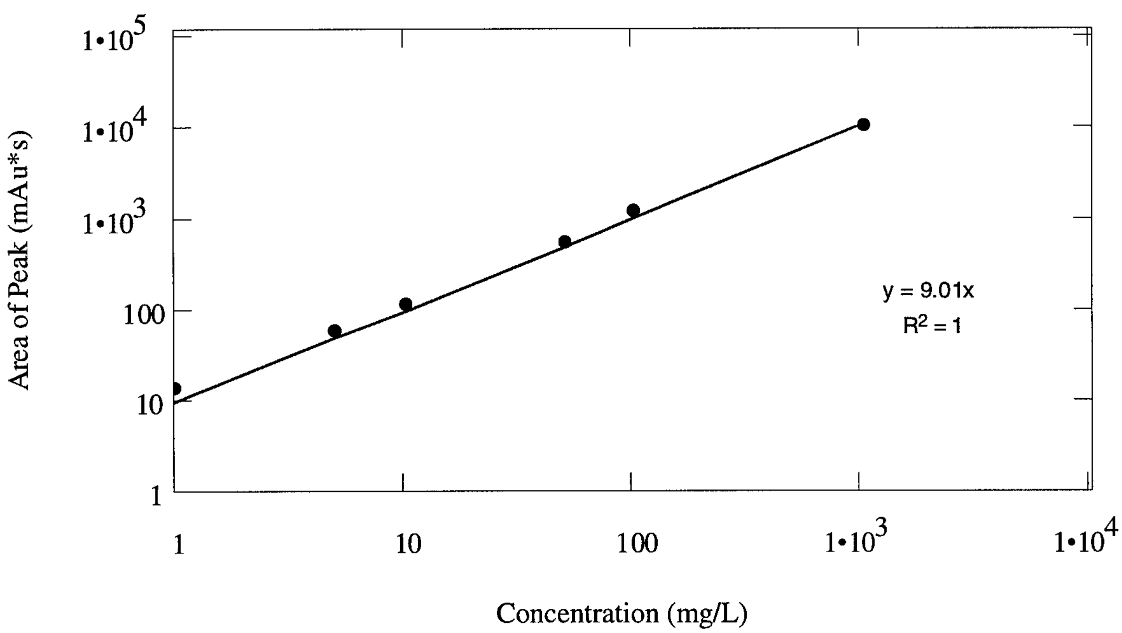
3.5.3 HPLC Detection Method for Tolyltriazole

After filtration of the samples, they were injected into a valve fitted 100 μm loop. The injection volumes were 10 μL, and the tolyltriazole was detected at wavelength of 280 ± 2 nm. The separation was carried out at room temperature (~22°C) with the diode array temperature set at room temperature (~22°C). The column used was an Altech® Adsorbanosphere C8 5U (250 mm x 4.6 mm). The mobile phase used two different solvents; a phosphate buffer composed of 0.5 mL phosphoric acid (H_3PO_4) and 0.65 gm potassium dihydrogen phosphate (KH_2PO_4) in one liter of de-ionized water, along with HPLC grade methanol. The solvents were set-up in a ratio and gradient that allowed for the tolyltriazole to peak at a reasonable time (8 min) and then flushed the column of any residual organics. The solvent ratio started at 30:70 (buffer:methanol) and transitioned to 50:50 (buffer:methanol) in the first 10 minutes, via the automated controls. At the 10 minute point, the ratio increased immediately to 10:90 (buffer:methanol) and stayed constant for the next 15 minutes in order to flush the organics from the system. The above method was used by Johnson (1997) and developed by PMC Specialties Group, Inc, of Cincinnati, Ohio.

3.5.4 Calibration Curve for HPLC Detection of Tolyltriazole

The concentrations used for establishment of the calibration curve were varied from 1 mg/L to 1,000 mg/L. The concentrations were prepared using the same tolyltriazole material with a base solution of methanol. The HPLC detection areas, identified as microabsorbency units * second (mAu*s), were calculated for each concentration (mg/L) with the HP Chem-station software. The calibration curve was then fitted with a linear regression line that possessed a $R^2 = 1.00$ (Pearson coefficient). Figure 3-2 depicts the calibration curve plotted in log/log scale for convenient interpretation and conversion of the HPLC detection areas (mAu*s) to concentrations (mg/L).

Figure 3-2
Calibration Curve for Tolyltriazole Detection with HPLC



Note: The limit of detection (LOD) was determined at ± 3mg/L. Appendix D lists all data and calculations for the calibration curve and LOD.

3.6 Microbial Colony Plate Count (MCPC)

3.6.1 Overview of MCPC Test

The method of microbial colony plate counting used a simple measurement of the number of living microbes and their health in soil. Theoretically, each healthy cell forms a single colony on the solid medium that can support its growth. After incubation, the number of colonies on the plate ideally equals the number of cells in the sample inoculated on the agar [McKane and Kandel, 121]. The plate counts must be sufficiently diluted prior to injection on the nutrient agar plate. The diluted sample provides sufficient area for colonies to grow separately. This allowed definitive counts of the individual populations. An overview of the test set-up is shown in Figure 3-3.

Figure 3-3
Overview of Microbial Colony Plate Count Test

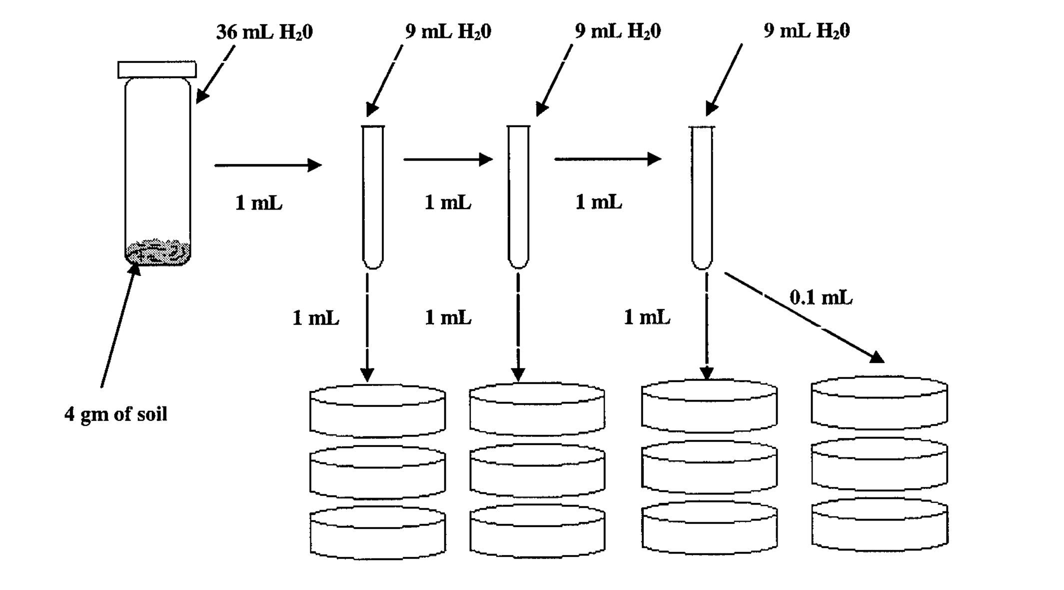


Diagram cited from McKane and Kandel, page 120

Three replicate MCPC tests (petri dishes) were preformed for each dilution. The MCPC method was applied to soils exposed to various concentrations of ADF

components. This helped determine the influence of the chemicals on the health and activity of the microbe populations.

3.6.2 Set-up of Materials for the MCPC Test

The preparation of nutrient agar plates followed *Standard Methods* protocols. The nutrient agar (Difco, Bacto™) was pre-sterilized at 121°C for 15 minutes in an autoclave. The dilution water was prepared with sodium chloride (NaCl) at 0.5 gm for one liter de-ionized water. This prevented the rupture of microbial cell membranes due to the osmotic pressure difference. The petri dishes (Fisher Brand, 95 x 15 mm) were pre-sterilized disposable-plastic. Incubation of the inoculated plates occurred for 2 – 3 days at 25°C in an incubator oven.

3.6.3 Counting Techniques for MCPC

Plates were examined at 12 hour intervals within the 2 – 3 day time period. The actual counting was done subjectively on a lighted colony counter (Leica™, Model 3327). The optimal time for the visual identification of microbial populations was at the 48 hr point. After 48 hours, the size and abundance of growths upon plates reduced the accuracy of counting. The ranges of normally accepted population counts on a plate is typically established between 30 – 300 individual colonies [Eaton *et al.*, 9-33].

3.7 Agar Well Diffusion Test (AWDT)

3.7.1 Overview of AWDT

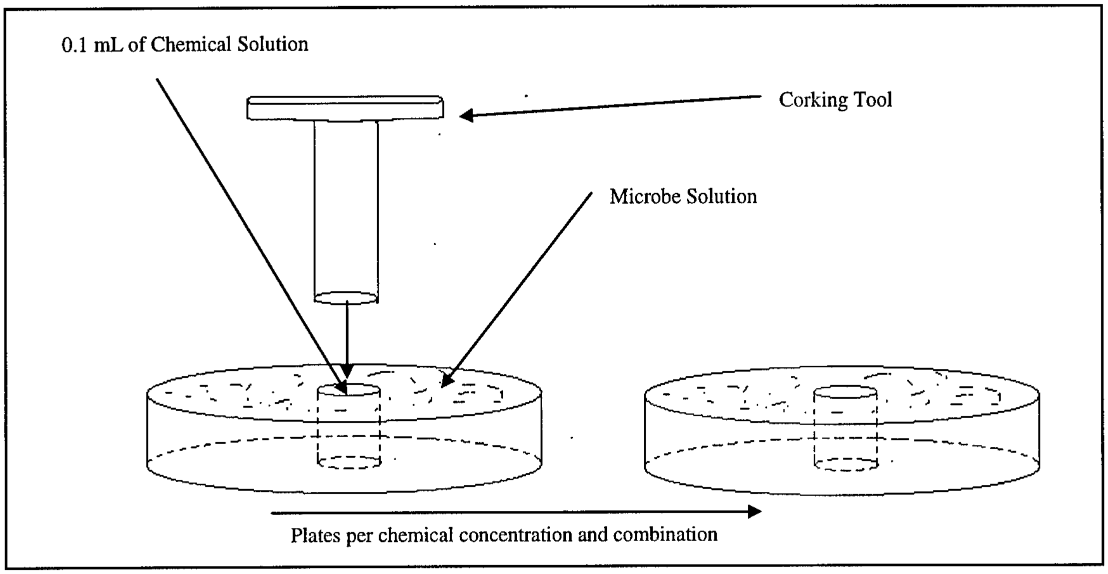
The agar well diffusion test is used to measure whether a chemical supports or

inhibits microbial growth and activity. The nutrient agar was used as a reliable food source to ensure a healthy population of microorganisms. A holding well was dug out of the agar media in the center of the prepared petri dish. Individual and combined ADF chemical solutions were prepared and placed in the well to allow diffusion onto the agar and newly introduced microorganisms. The microbes were allowed to incubate and interact with the chemicals. The inhibition or proliferation of microbial colonies around the well was used to measure toxicity. If microbes exist in and around the well area, then the chemical concentration is apparently not toxic to them. If microbial colonies formed a measurable distance away from the well area, then toxicity is apparent. A toxicity test similar to this is described in the *Handbook of Environmental Microbiology* [Mills, 355].

3.7.2 Set-up of Materials for the AWDT

Nutrient agar is prepared within an autoclave at 121°C for 15 minutes as described above in section 3.6.2. The agar was then poured into pre-sterilized petri dishes and allowed to solidify for one hour. Using a "corking tool" (pre-sterilized) a small well was placed in the center of the agar. A microbial rich solution is prepared and spread upon the plate surface. Individual and combined ADF chemical solutions of propylene glycol (10,000 mg/L), tolyltriazole (5,000 – 10,000 mg/L), or propylene glycol (10,000 mg/L) with tolyltriazole (5,000 – 10,000 mg/L) were prepared and used to fill (~0.1 mL) the well. The layout of ADF chemical concentrations and combinations is located in Appendix J. The petri dishes incubated at 25°C for several days and were monitored for signs of toxicity around the well on a 12 hour basis. The AWDT used several plates per chemical treatment. See Figure 3-4 for an overview of the layout.

Figure 3-4
Overview of Agar Well Diffusion Test



3.8 Statistical Methodology

The first research objective was to determine the impact on microbial biodegradation of individual ADF chemicals on an uncontaminated soil environment. This determination was made using the O_2 consumption totals of the contaminated soil (PG alone or TTA alone) against the uncontaminated soil (blank soil). A two-sample t-test was used to measure the difference of O_2 total means (chemical treatment on soil minus the blank) using a significance level of $\alpha = 0.05$. The null hypothesis was that there was no effect on O_2 consumption due to contaminates addition. The t-test results were converted into a 95% confidence interval (CI) for the entire respirometry run period (336 hrs). The CI was graphed to provide a visual explanation of increased O_2 consumption (biodegradation) or decreases (inhibition). Appendix F contains a detailed layout of the statistical set-up, formulas, and figures.

The second research objective was to determine the impact on microbial

biodegradation due to the combined ADF chemical treatment (PG & TTA) on an uncontaminated soil environment. The null hypothesis states that there was no difference in O_2 consumption due to combined ADF components compared against the individual ADF components on uncontaminated soil. This determination was made using the mean O_2 consumption totals of the contaminated soil (PG & TTA) against a linear combination of individual treatments (PG alone, TTA alone, and blank) on uncontaminated soil. A two-sample t-test was used to measure the difference of mean O_2 totals using a significance level of $\alpha = 0.05$. The t-test results were converted into a 95% CI for the entire respirometry run period (336 hrs). The CI provided a visual depiction for the amount of O_2 consumption increases or decrease due to the combined ADF components. See Appendix G for a detailed layout of the statistical set-up, formulas, and figures.

The third research objective was to compare ADF pre-treatment/pre-conditioning of the same soil for biodegradation activity. This objective was checked with the initial O_2 consumption rates (using ThOD calculations to develop the initial biodegradation rates) from propylene glycol (1,000 mg/kg) application on uncontaminated soil (unconditioned microbes) against pre-contaminated soil (microbes acclimated to propylene glycol). The statistical test method used a two-sample t-test with a significant level of $\alpha = 0.05$. The null hypothesis was that there was no difference between the initial biodegradation rates of acclimated soil compared to uncontaminated soil once propylene glycol (1,000 mg/kg) was applied. See Appendix L for a detailed layout of the statistical test method.

IV. Data Analysis

4.1 Overview of Data Analysis

Two forms of analyses were performed on the data; visual and statistical. Visual and statistical analyses were conducted on both phase-one and phase-two respirometry data. Statistical tests were done on HPLC results and visual analysis was conducted on both the MCPC and the AWDT data.

4.2 Repeatability/Consistency of Laboratory/Respirometry Procedures

A comparison/review of all six experimental runs was performed prior to analyzing the respirometry data for biodegradation effects. The goal was to show consistency and repeatability of the respirometer/laboratory procedures used throughout experimental runs that comprised the research. Once accuracy/quality was assured in the respirometer measurements and proper laboratory techniques, the focus moved to analyzing the data for microbial affects from the ADF components.

The checks for respirometry measurements and laboratory procedures used a comparison of similar treatments within the respirometry runs. The statistical tests were performed with a one-way analysis of variance (ANOVA) using a P-value and F-test. The one-way ANOVA results were then used to generate a Tukey-pairwise test of the mean O_2 consumption totals for each respirometry run. This was used to identify any possible irregularities in respirometry runs.

There were two specific soil treatments replicated in the experiments. First, de-ionized H_2O (blank) was used in three runs. Then 1,000 mg/kg of propylene glycol

(PG_{1000}) was used in five runs. The repeatability and performance of the respirometer were performed through comparison of blank treatments on uncontaminated soil. Consistency in laboratory procedures and techniques was determined through the PG_{1000} treatments used in respirometry runs. If preparation of solutions were incorrectly performed, then a significant difference in O_2 consumption would develop, thus eliminating the respirometry run from analysis.

The cumulative O_2 consumption totals (μL) at the 288 hr point, for both blank and PG_{1000} treatments, were obtained from all respirometry runs. The statistical tests for each soil treatment were generated with STATISTIX® 4.0 software using a significance level of $\alpha = 0.05$. The null hypothesis stated that for the replicated test conditions, there was no difference in respirometry runs (mean O_2 consumption totals, 288 hr point).

4.2.1 Statistical Test of Blank Respirometry Runs for Repeatability/Consistency

There were three microcosm bottles in each of the three runs to compare. The O_2 consumption totals for each respirometry run were compared for outliers, using a Box and Whiskers plot. The plot showed no outliers. The residuals for each respirometry run were calculated and plotted on a Wilk-Shapiro/Rankit plot of residuals. The data appeared to have aptness from the Wilk-Shapiro statistic = .853 (acceptable).

An F-test value and P-value were determined from the one-way ANOVA. The results of the tests are summarized in Table 4-1.

Table 4-1
One-way ANOVA results for De-ionized H_2O on Uncontaminated Soil (288 hr point)

Test	Testing Values (Devore, 709)	Test Results STATISTIX 4.1	Results
$f^* > F_{crit}$ Reject Null	$F_{crit} = 5.14$	$f^* = 0.69$	Do not reject the Null
P < alpha Reject Null	alpha = 0.05	P = 0.5339	Do not reject the Null

See Appendix M, page M-3 for results

The null hypothesis was not rejected, thus stating the blank (de-ionized H_20) soil treatments have shown that the respirometer maintained repeatable/consistent measurements. Table 4-2 contains the Tukey-pairwise comparison of means from the one-way ANOVA results.

Table 4-2
Consistency of Respirometry Runs using a Tukey-pairwise Comparison of O_2 Mean Totals (Blank on Uncontaminated Soil, 288 hr point)

RUN	MEAN	HOMOGENEOUS GROUPS
1	8264	I
2	8048	I
3	7881	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

The comparison (Table-4-2) shows consistency in all the O_2 mean totals tested and confirms the F-test and P-value acceptance of the null hypothesis (Table 4-1).

4.2.2 Statistical Test of PG_{1000} Respirometry Runs for Repeatability/Consistency

Three to five microcosm bottles were compared in each of the five runs. The O_2 consumption totals at the 288 hr point for respirometry runs were compared for outliers, using a Box and Whiskers plot. The plot showed no outliers. The residuals for each

respirometry run were calculated and plotted on a Wilk-Shapiro/Rankit plot of residual. The data appeared to have aptness from the Wilk-Shapiro statistic = .995 (acceptable).

An F-test value and P-value were obtained from the one-way ANOVA. The degrees of freedom were calculated and the F-critical (F_{crit}) value was determined. The results of the tests are summarized in Table 4-3.

Table 4-3
One-way ANOVA results for PG_{1000} on Uncontaminated Soil (288 hr point)

Test	Testing Values (Devore, 709)	Test Results STATISTIX 4.1	Results
$f^* > F_{crit}$ Reject Null	$F_{crit} = 2.87$	$f^* = 54.87$	Reject the Null
P < alpha Reject Null	alpha = 0.05	P = 0.000	Reject the Null

See Appendix M, page M-5 for results

The null hypothesis was rejected, thus stating the propylene glycol soil treatment/runs have shown inconsistency. This prompted the completion of a Tukey-pairwise comparison of means from the one-way ANOVA results, shown in Table 4-4.

Table 4-4
Consistency of Respirometry Runs using a Tukey-pairwise Comparison of O_2 Mean Totals (PG_{1000} on Uncontaminated Soil, 288 hr point)

RUN	MEAN	HOMOGENEOUS GROUPS	
2	44873	I	
1	37551		I
5	37265		I
4	36837		I
3	35803		I

THERE ARE 2 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

The comparison (Table 4-4) shows inconsistency in the mean O_2 consumption totals for Run-2, compared with the other respirometry run means. This supported the

removal of this data set. This infers that the laboratory procedure might have been compromised. The error might have been in the preparation of the propylene glycol solution. A higher concentration (greater than $\geq$10,000 mg/L) solution might have been prepared, thus causing the higher O_2 consumption totals.

In addition, Run-2 had been cut short at 288 hr point due to a power failure. This would have restricted the use/comparison of other respirometry runs/data that had operated for a full 336 hours in the research. This supported re-accomplishment of Run-2, and removing the old Run-2 data that was questionable.

After Run-2 was re-accomplished, a new statistical test was performed to check the consistency in laboratory procedures. The 288 hr time period for O_2 consumption totals were compared for outliers, using a Box and Whiskers plot. The plot showed no outliers. The residuals for each respirometry run were calculated and plotted on a Wilk-Shapiro/Rankit plot of residuals. The data appeared to have aptness from the Wilk-Shapiro statistic = .936 (acceptable).

An F-test value and P-value were provided from the one-way ANOVA results. The degrees of freedom were calculated and the F-critical value was determined. The results of the tests are summarized in Table 4-5.

Table 4-5
One-way ANOVA results for PG_{1000} on Uncontaminated Soil (288 hr point)

Test	Testing Values (Devore, 709)	Test Results STATISTIX 4.1	Results
$f^* \geq F_{crit}$ Reject Null	$F_{crit} = 2.87$	$f^* = 2.75$	Do not reject the Null
$P \leq$ alpha Reject Null	alpha = 0.05	P = 0.0649	Do not reject the Null

See Appendix M, page M-8 for results

The null hypothesis was <u>not rejected</u>, thus stating the propylene glycol soil treatment/runs have shown consistency. This prompted the completion of a Tukey-pairwise comparison of means from the one-way ANOVA results as shown in Table 4-6.

Table 4-6
Consistency of Respirometry Runs using a Tukey-pairwise Comparison of O_2 Mean Totals (PG_{1000} on Uncontaminated Soil, 288 hr point)
(Run-2, re-accomplished and included)

RUN	MEAN	HOMOGENEOUS GROUPS
1	37551	I
5	37265	I
4	36837	I
2	36205	I
3	35803	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

The incorporation of the new Run-2 data set has shown no significant difference amongst all the data sets (respirometry runs).

4.2.3 Summary of Respirometry Data for Repeatability and Consistency

Overall, the comparison of O_2 results for all 2400+ respirometer run hrs (48,000+ microcosm hrs) showed consistency. This consistency is found in the comparison of background soil respiration and other similar treatments that were used throughout all six respirometry runs performed. Repeatability has definitely improved by following the recommendations of Johnson (1997) and O'Malley (1997). Other experiments by Thomas (1996), Totten (1995), and Baker (1995) also confirm the precision and accuracy of this particular respirometer.

4.3 Biodegradation Analysis of Respirometry Data (Phase-one)

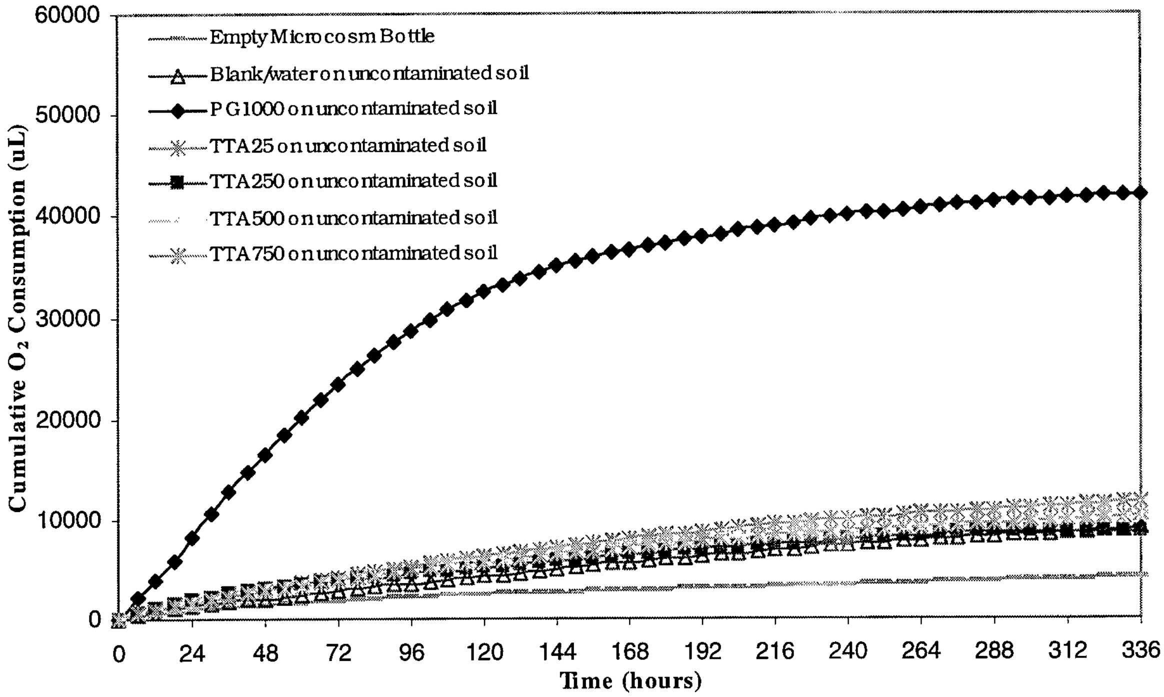
Respirometry work in phase-one used uncontaminated soils (unconditioned microorganisms). The uncontaminated media allowed measurements of microorganisms' initial response to the ADF's chemical components. The statistical tests were designed to determine if any effect (inhibition, biodegradation, or no effect) of O_2 consumption totals occurred due to the individual and combined ADF chemical treatments on soil. The procedures for statistical testing of individual ADF component treatments are summarized in Appendix F, and the combined ADF component treatments are summarized in Appendix G.

Biodegradation was measured through O_2 consumption and CO_2 production. Consumption and production activities were measured by recording accumulated totals (μL) and rates (μL/hr). CO_2 production mirrored O_2 consumption, consequently only O_2 data was analyzed. A representative collection of all plotted forms (μL and μL/hr) of O_2 and CO_2 data are found in Appendix E for respirometry Run-1 (see Figures E-1 through E-5).

4.3.1 Analysis of Individual ADF Component Treatments on Uncontaminated Soil

Figure 4-1 plots cumulative O_2 consumption measurements for the individual ADF chemical treatments on uncontaminated soil for phase-one. All ADF treatments lines depicted in the figure are an average of five microcosms and blank treatment lines are an average of three microcosms. Refer to Appendix E for the original data from respirometry runs (Run-1, Run-2, Run-3, and Run-5) related to Figure 4-1.

Figure 4-1
Cumulative O_2 Consumption (μL) for
Individual ADF Components on Uncontaminated Soil



Note: legend designation TTA25 (or others) refers to TTA_{25} or 25 mg/kg tolyltriazole

Figure 4-1 demonstrated a higher cumulative O_2 consumption for propylene glycol compared to any of the tolyltriazole concentrations on soil. The figure also demonstrated when TTA_{25}, TTA_{250}, TTA_{500}, or TTA_{750} were placed on uncontaminated soil, the respiration totals were about the same as the blank treatment on uncontaminated soil.

The respirometry data for the rate of O_2 consumption was assembled from all the phase-one runs (Run-1, Run-2, Run-3, and Run-5) in Figure 4-2. All ADF treatment lines depicted in the figure are an average of five microcosms and the blank treatment lines are an average of three microcosms. Appendix E contains original respirometry runs related to Figure 4-2.

Figure 4-2
Rate of O_2 Consumption (μL/hr) for Individual ADF Components
on Uncontaminated Soil

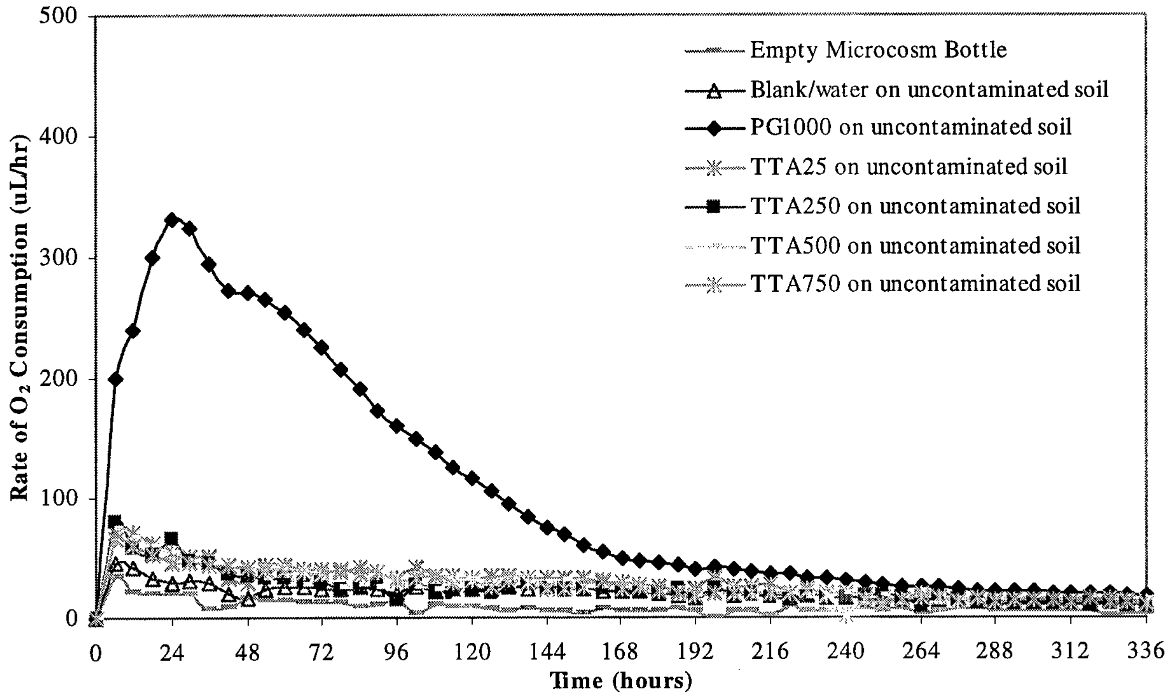


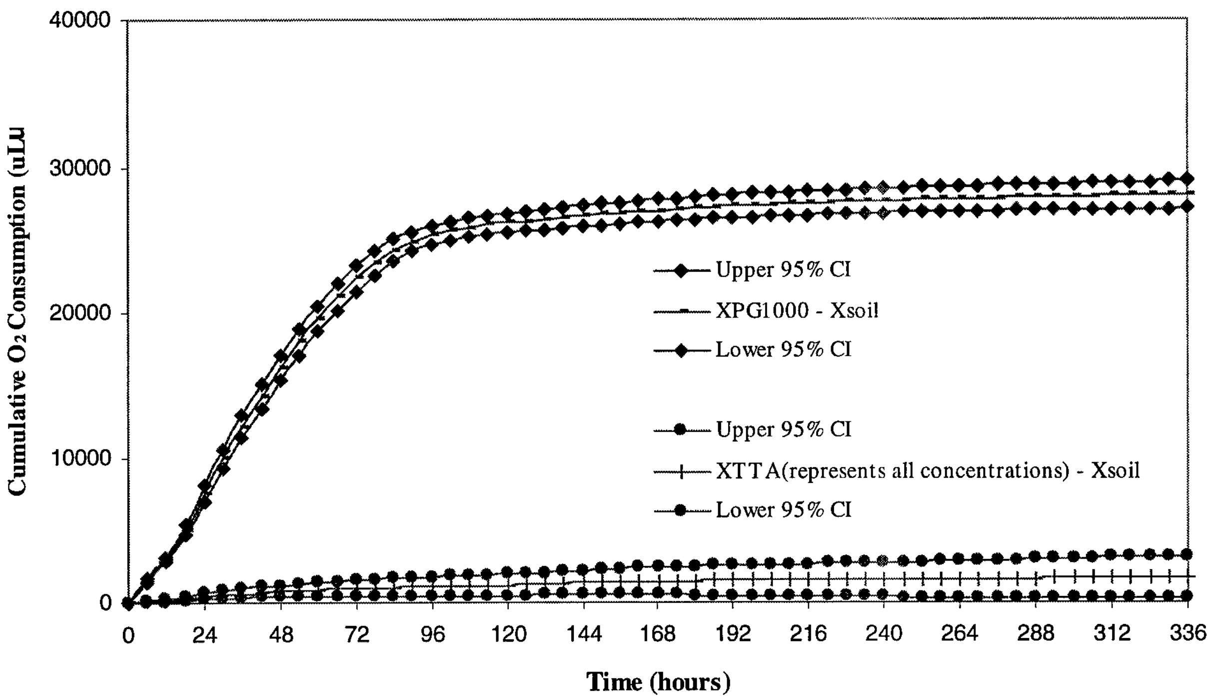
Figure 4-2 demonstrated O_2 consumption for PG_{1000} had returned to blank soil treatment levels after the 264 – 336 hr point, while the $TTA_{25\text{-}1000}$ treatments were similar to blank soil respiration activity.

Statistical tests were then applied to the cumulative O_2 consumption totals to determine if the individual ADF components (PG alone or TTA alone) were greater than the blank soil treatment. The statistical tests were followed from Johnson's (1997) approach. The null hypothesis was that there was no effect on the O_2 consumption due to the contaminant addition compared to O_2 consumption of blank soil. Biodegradation was supported when there was a significant difference in the O_2 consumption for chemical treatment on soil against the blank treatment on soil [Johnson, 4-30]. The evaluation of biodegradation, inhibition, or no effect was produced through a two-tailed t-test, with a

significance level of $\alpha = 0.05$, at each of the 6 hour sampling intervals over the entire respirometry period. The results are found in Table F-1 through Table F-5.

A 95% CI was developed from the t-test results to visually depict the size of the difference in the O_2 consumption effects. If the CI hooked the zero line of the y-axis, then the null hypothesis was supported. If the lower CI was above the zero line of the y-axis, then significant O_2 consumption (biodegradation) was supported. While if the upper CI was the zero line of the y-axis then inhibition was supported. Figure 4-3 summarizes the 95% CI results found in Appendix F.

Figure 4-3
Statistical Tests on Cumulative O_2 Consumption Totals (μL)
for Individual ADF Chemical Components on Uncontaminated Soil



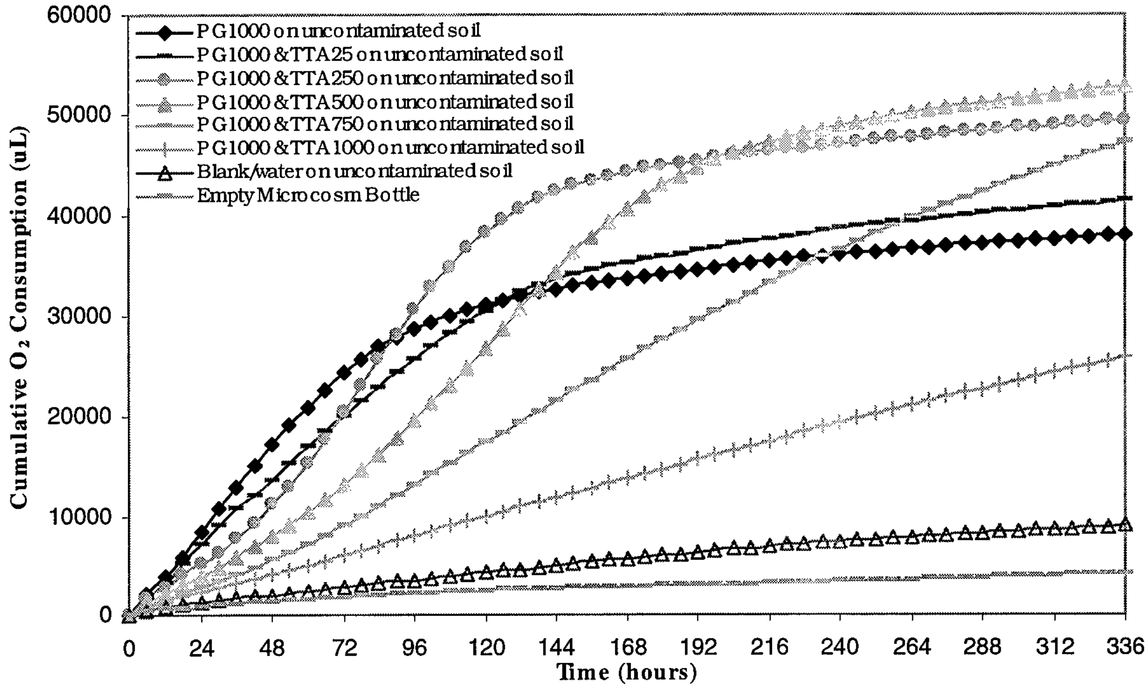
Note: Appendix F contains data referenced in Figure 4-3

The results showed that PG_{1000} 95% CI (top lines) did not hook the zero line of the y-axis. Therefore the 95% CI indicates PG_{1000} does consume O_2 above blank soil levels. This supports the potential biodegradation of propylene glycol alone in soil. A representative tolyltriazole CI was developed to represent the $TTA_{25\text{-}750}$ CI's (due to the overlap of the lines) and to establish a reference for the PG_{1000} CI. The TTA_{25} and TTA_{250} 95% CI did hook the zero line of the y-axis, indicating no significant difference (no effect) in O_2 consumption occurred. However, there was additional O_2 consumption compared with blank soil respiration for TTA_{500} and TTA_{750}. This indicated some potential biodegradation of tolyltriazole alone in soil.

4.3.2 Analysis of Combined ADF Component Treatments on Uncontaminated Soil

Varied concentrations of tolyltriazole (25 – 1,000 mg/kg) were combined with a fixed concentration of propylene glycol (1,000 mg/kg) to determine if there were any effects on O_2 consumption (biodegradation). Figure 4-4 combines cumulative O_2 consumption measurements from all phase-one respirometry runs (Run-1, Run-2, Run-3, and Run-5). The ADF treatment lines depicted in Figure 4-4 are an average of five microcosms and the blank treatment lines are an average of three microcosms. Appendix E contains original respirometry runs related to Figure 4-4.

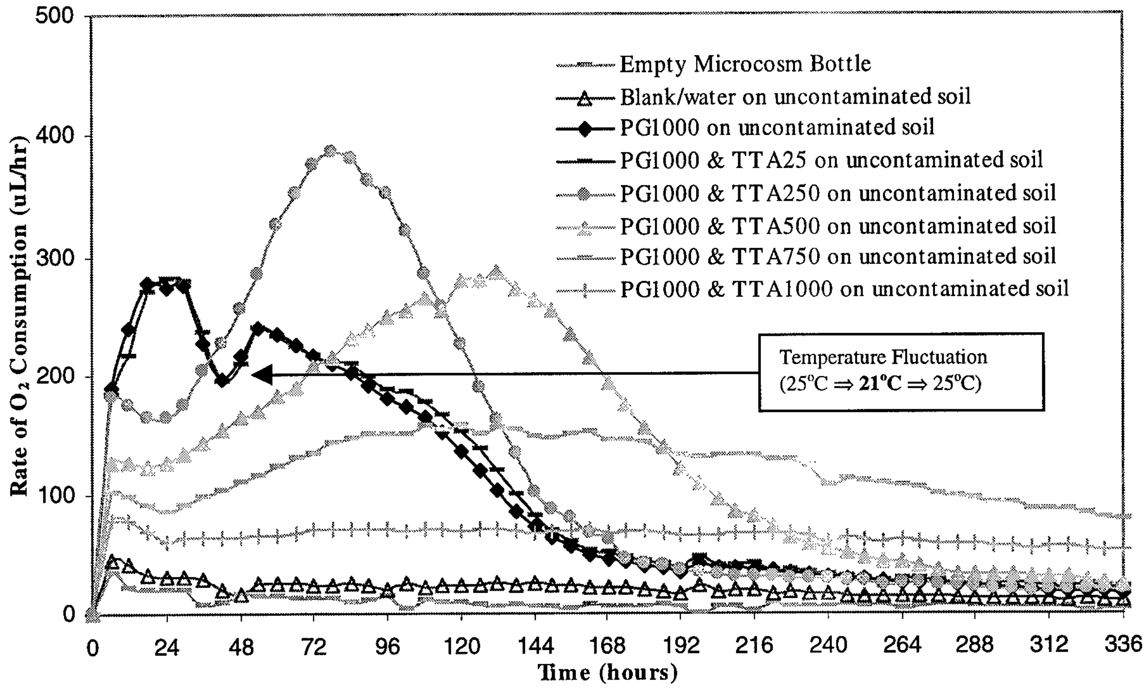
Figure 4-4
Cumulative O_2 Consumption (µL) for Combined ADF Chemical Components on Uncontaminated Soil



The data in Figure 4-4 above, demonstrated that for mixtures of increasing $TTA_{25 \Rightarrow 750}$ with a fixed PG_{1000}, the total accumulated O_2 consumption totals (336 hr point) increased compared to the a PG_{1000} only treatment on soil. Figure 4-4 also demonstrated that the PG_{1000} & TTA_{1000} consumption totals were lower then PG_{1000} only treatment on soil, due mainly to the reduced respiration activity seen in the rates of O_2 consumption.

Figure 4-5A and Figure 4-5B depicts the rate of O_2 consumption for the combined ADF components on uncontaminated soil from all the phase-one respirometry data. The plot lines in Figure 4-5A used an average of five microcosms for each ADF treatment, and three microcosms for the blank soil treatment.

Figure 4-5A
Rate of O_2 Consumption (μL/hr) for Combined ADF Components
on Uncontaminated Soil

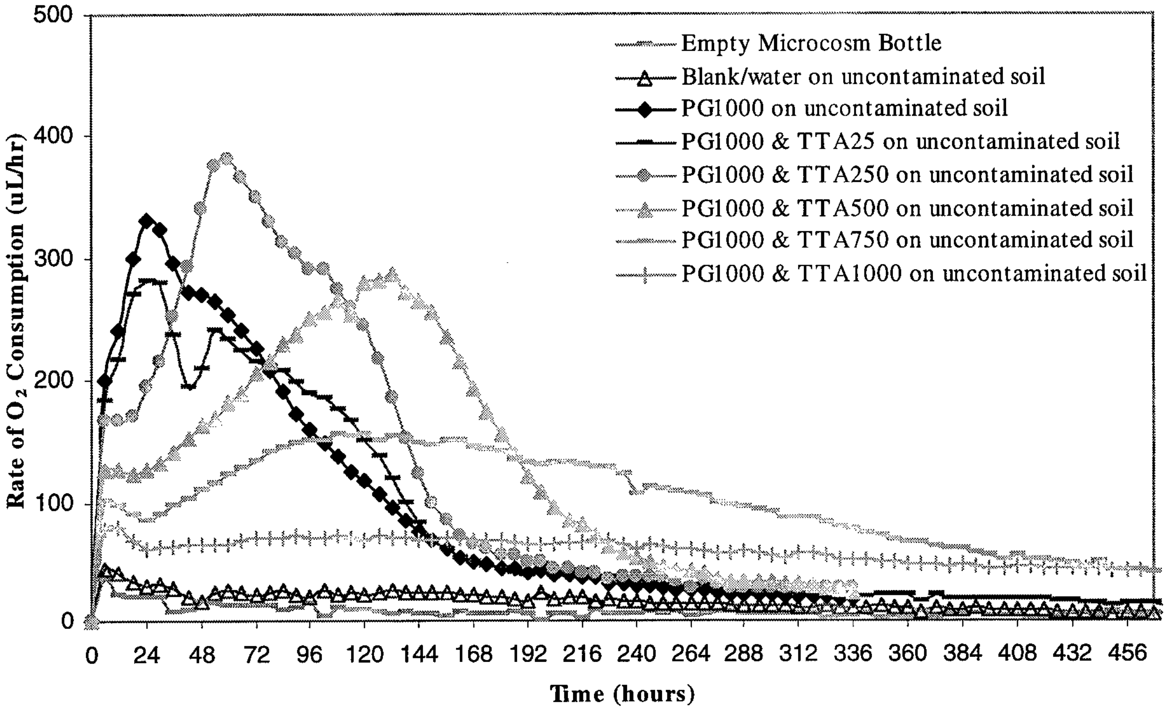


Note: The temperature fluctuation (PG_{1000} & TTA_{25}) reduced respiration activity for a limited time.

In Figure 4-5A, the PG_{1000} and the PG_{1000} & TTA_{25} data lines were produced strictly from Run-1 data. The importance of this detail was to depict the minimal difference in the rates of O_2 consumption for the two treatments (PG_{1000} and PG_{1000} & TTA_{25}).

In Figure 4-5B, an average of 15 microcosms (Run-1, Run-2, and Run-3) were used to depict the PG_{1000} plot line, along with five microcosms for the other ADF treatments, and three microcosms for blank soil treatments. The time scale of the y-axis was also extended from 336 hrs to 468 hours. The longer time period enhanced the depiction of PG_{1000} & TTA_{750} and PG_{1000} & TTA_{1000} slowed rate of O_2 consumption.

Figure 4-5B
Rate of O_2 Consumption (μL/hr) for Combined ADF Components
on Uncontaminated Soil



Both Figures 4-5A and Figure 4-5B demonstrated the slowing rate of O_2 consumption with the increasing concentration of $TTA_{25\Rightarrow1000}$ combined with PG_{1000}. Even at the 468 hr point, the rate of O_2 consumption for the mixture of PG_{1000} & TTA_{750} and PG_{1000} & TTA_{1000} had not returned to the rate of O_2 consumption rate for blank soil.

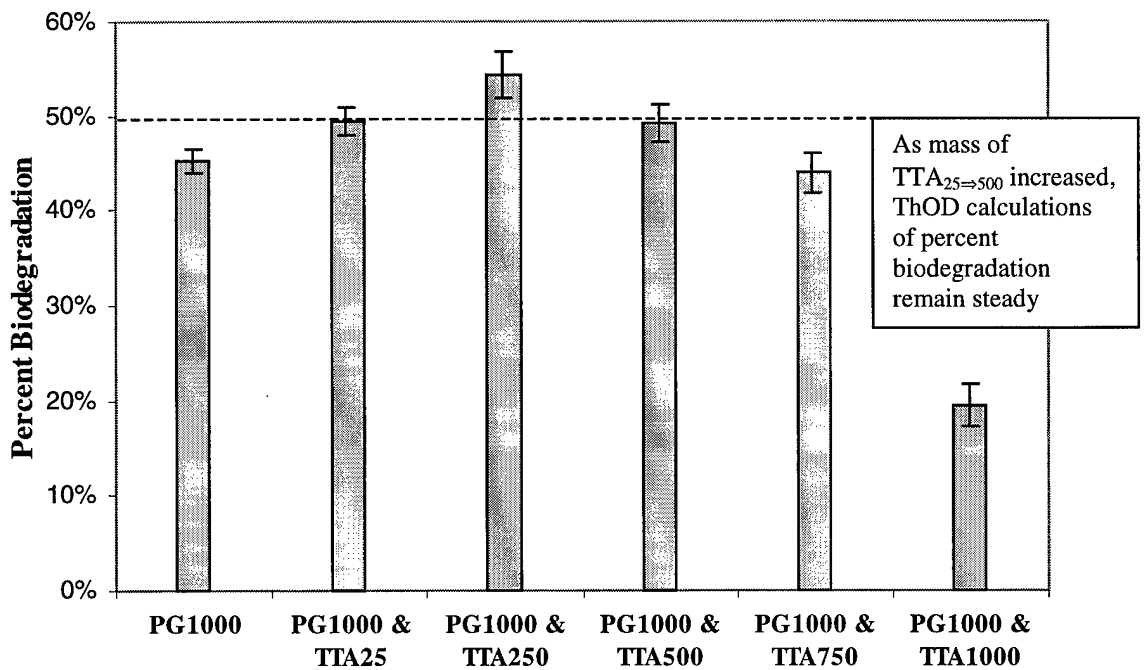
ThOD equations for propylene glycol and tolyltriazole (section 2.3.5 and 2.3.7, respectively) were then applied to the observed effect (respirometry data) of increased O_2 consumption due to the increased mass $TTA_{25\Rightarrow1000}$ with a fixed mass of PG_{1000} (Figure 4-5). The focus was on whether the apparent increase in O_2 consumption was proportional/correlated to the ThOD of ADF chemicals potential biodegradation in soil (PG_{1000} & $TTA_{25\Rightarrow1000}$). The "total" ThOD was calculated for the available mass of ADF

chemicals in the uncontaminated soil. The "total" ThOD results were then converted from mass (mg) O_2 to volume (μL) O_2, using the Ideal Gas Law.

The "actual" O_2 consumption totals (μL) were collected from the various treatments (PG & TTA) where the rate of O_2 consumption had returned to blank soil respiration rates, typically around the 336 – 468 hour point. The term "actual" O_2 consumption total equals the O_2 consumption total of the ADF soil treatment minus the O_2 consumption total of the blank soil treatment.

A percent biodegradation for available ADF components in soil was then calculated from the "actual" O_2 consumption total (μL) divided by the "total" ThOD (μL). Appendix K contains the data and calculations for the percent biodegradation shown in Figure 4-6.

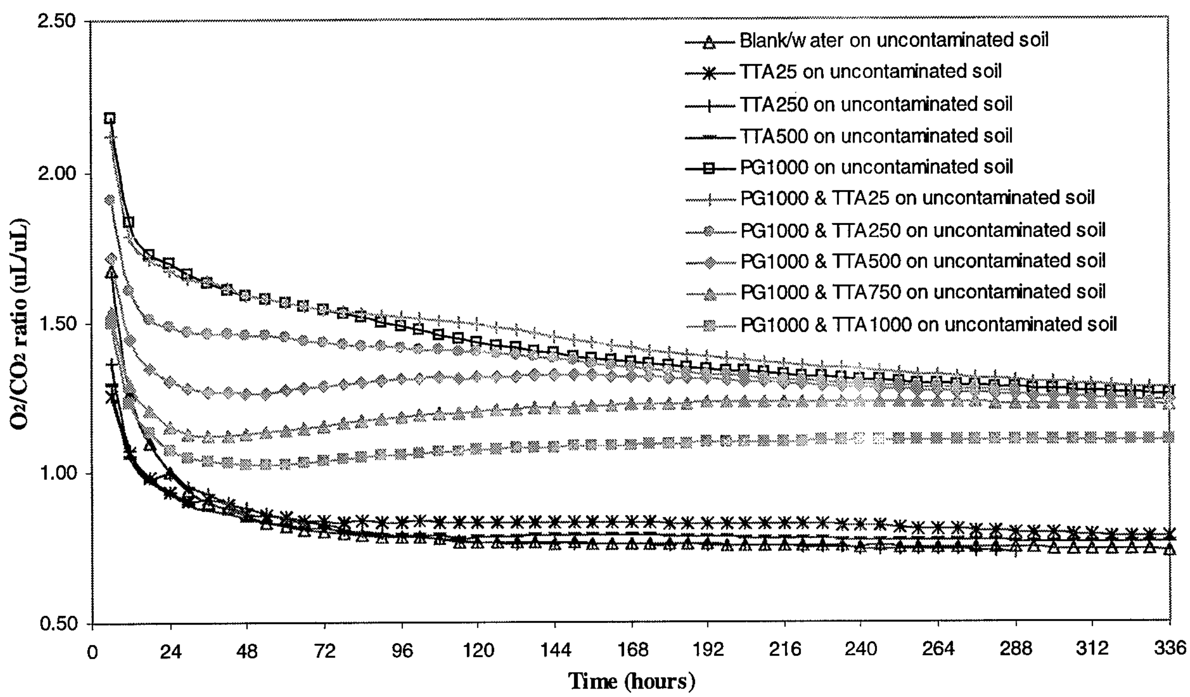
Figure 4-6
Percent Biodegradation from ThOD of Available ADF Chemical Components on Uncontaminated Soil



The column graph demonstrated an approximately steady biodegradation percent (~50%) for a varied mass of $TTA_{25\Rightarrow1000}$ with a fixed mass of PG_{1000} in soil. PG_{1000} & TTA_{750} might also have achieved 50% biodegradation if the O_2 respiration activity had returned to blank soil respiration activity (uncompleted O_2 consumption). Note, the ThOD calculations for the percent biodegradation represent microbial respiration/activity for degrading the food source in an aerobic environment.

Figure 4-7 summarizes all of the respiration exchange ratios (RER's = O_2/CO_2 in units of $\mu L/\mu L$) for all of the phase-one respirometry runs.

Figure 4-7
O_2/CO_2 Ratios for All Phase-one Data



Overall, as the concentration of tolyltriazole increased with propylene glycol in soil, the overall RER's became lower. Perhaps the lowering RER's with increasing tolyltriazole concentrations was correlated to the ThOD calculations. The RER's were

calculated from the stochemetric equation from the ThOD calculations for propylene glycol and tolyltriazole (section 2.3.5 and 2.3.7, respectively). The two different ThOD RER's for propylene glycol and tolyltriazole were weighted with the amount of available chemical in the soil (Table 4-7).

Table 4-7
Weighted ThOD RER's from Available ADF Components in Soil Treatments

		Treatment(s) of Propylene Glycol and Tolyltriazole in Soil					
ThOD ratio for O_2/CO_2		PG_{1000} &	TTA_0	PG_{1000} &	TTA_{500}	PG_{1000} &	TTA_{1000}
Table 2-1	PG = 1.333	1000/1000 * 1.33		1000/1500 * 1.33		1000/2000 * 1.33	
Table 2-2	TTA = 0.9285		0/1000*.929		500/1500*.929		1000/2000 * .929
Averaged O_2/CO_2 ratio =			1.333		1.198		1.130

Thus, a decreasing ThOD RER's would occur as calculated in Table 4-7 and might support the decreasing RER's seen in Figure 4-7.

A statistical test was conducted to identify the change on microbial respiration activity due to the combined ADF chemical treatment (PG & TTA) compared to individual ADF components (PG alone and TTA alone) on uncontaminated soil. The null hypothesis stated there was no difference in O_2 consumption due to combined ADF components compared to the individual ADF components on uncontaminated soil. This determination was made using O_2 consumption totals of the contaminated soil (PG & TTA) against a linear combination of individual treatments (PG alone, TTA alone, and blank) on uncontaminated soil. Appendix G contains a visual explanation of this linear combination. A two-sample t-test was used to measure the difference of O_2 total means using a significance level of $\alpha = 0.05$. Figure 4-8 depicts the set-up of the O_2 means totals to perform the t-test in the upcoming CI results (Figure 4-9).

The t-test results were converted into a 95% CI for the entire respirometry run

period (336 hrs). The CI provided a visual depiction of the amount O_2 increased or decreased due to the combined ADF components compared to the individual effects of the ADF components. The null hypothesis was based around the zero line of the y-axis. Appendix G contains a detailed layout of the statistical set-up, formulas, and Figures G-1 through G-5. Figure 4-8 overlaid three statistical tests (PG_{1000} & TTA_{25}, PG_{1000} & TTA_{500}, and PG_{1000} & TTA_{750}) to show the differences in O_2 consumption effects from the combination of ADF components.

Figure 4-8
Difference Between the Means (O_2) using a 95% Confidence Interval for the Linear Combination of Tolytriazole and Propylene Glycol on Uncontaminated Soil

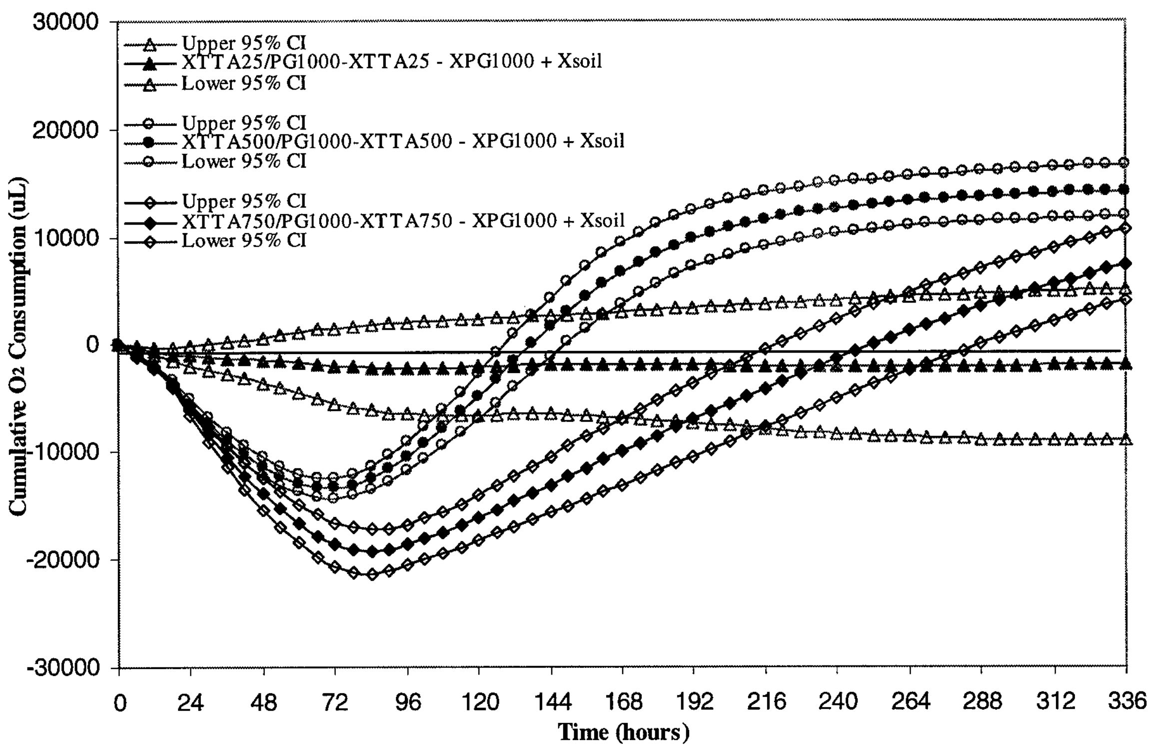


Figure 4-8 revealed no significant difference in O_2 consumption when TTA_{25} was combined with PG_{1000}, since the 95% CI hooked the mean of the zero line of the y-axis (null hypothesis). The other comparison of PG_{1000} & TTA_{500} and PG_{1000} & TTA_{750}

showed significant O_2 consumption effects due to the combination of propylene glycol and tolyltriazole in soil. The 95% CI reveals inhibition on O_2 consumption for the first 140 hrs, since PG_{1000} & TTA_{500} are below the zero, while PG_{1000} & TTA_{750} showed inhibition for the first 252 hrs.

These lags indicate unusual inhibition effects as the concentration of tolyltriazole increased with propylene glycol. As explained by Johnson (1997), the process of biodegradation usually begins after a lag period in which microorganisms are adjusting to the new contaminate(s) by producing needed enzymes. Populations that cannot handle a certain chemical and concentration might die off, and new populations will emerge in their place. The statistical test only confirms the unusual O_2 consumption activity.

4.3.3 HPLC Analysis of Tolyltriazole Residual in Spent Soil

HPLC analysis of tolyltriazole concentrations/residuals was performed before respirometry runs (without biodegradation pathway), and immediately after the respirometry runs (potential biodegradation pathway). The preparation of HPLC calibration curves for tolyltriazole detection is outlined in Appendix C. The methodology section (see page 3-18) contains the preparation of soil samples and the extraction processed used for measuring the tolyltriazole for HPLC analysis.

The HPLC calculations of percent degradation are found in Appendix H, and are summarized in Table 4-8A.

Table 4-8A
Percentages of Tolyltriazole Residual Recovered

Treatment	Percent of tolyltriazole residual measured through HPLC analysis					
	Before Respirometry Test (3 samples used)			After Respirometry Test (5 microcosms used)		
	Avg	Std Dev	Reference	Avg	Std Dev	Reference
TTA_{25}	99.79%	1.35%	Table H-4	48.97%	5.05%	Table H-5
TTA_{250}	90.56%	0.33%	Table H-4	81.51%	3.89%	Table H-6
TTA_{500}	95.15%	0.08%	Table H-4	No test performed	-------	-------
PG_{1000} & TTA_{25}	97.21%	1.17%	Table H-4	40.17%	3.73%	Table H-5
PG_{1000} & TTA_{250}	95.59%	0.17%	Table H-4	73.43%	3.23%	Table H-6
PG_{1000} & TTA_{500}	95.93%	0.12%	Table H-4	No test performed	-------	-------

Note: No HPLC tests were performed on spent respirometry soil from Run-3 (TTA_{500} and PG_{1000} & TTA_{500}) due to use in the phase-two experiments.

The tolyltriazole percent recovered before respirometry runs showed that the majority was recovered (90 – 99%), with or without the presence of propylene glycol, when immediately extracted from the soil. The results are not necessarily a good baseline to compare for potential biodegradation after the respirometry. There are too many degradation pathways to account for the loss of tolyltriazole (18 – 60%) when in contact with the soil (two weeks). These unknown degradation pathways were things such as the potential for strong absorption of the chemicals to the soil, physical change of the chemicals, or biotic reaction to the chemicals.

However, specific attention was placed on the additional degradation of tolyltriazole when in the presence of propylene glycol. This attention was supported by the respiration data, which had shown a larger O_2 consumption totals (μL) for the combination of propylene glycol and tolyltriazole compared to propylene glycol alone (as supported in Figure 4-6).

A pattern of additional degradation was observed for the mass of tolyltriazole when present with propylene glycol, as shown in Table 4-8B.

Table 4-8B
Percentages of Tolyltriazole Residual Recovered

Treatment	Percent of tolyltriazole residual measured through HPLC analysis After Respirometry Test (5 microcosms used)	
	Avg	Std Dev
TTA_{25}	48.97%	5.05%
TTA_{250}	81.51%	3.89%
TTA_{500}	No test performed	-------
PG_{1000} & TTA_{25}	40.17%	3.73%
PG_{1000} & TTA_{250}	73.43%	3.23%
PG_{1000} & TTA_{500}	No test performed	-------

8.8% Δ ± Std Dev

8.1% Δ ± Std Dev

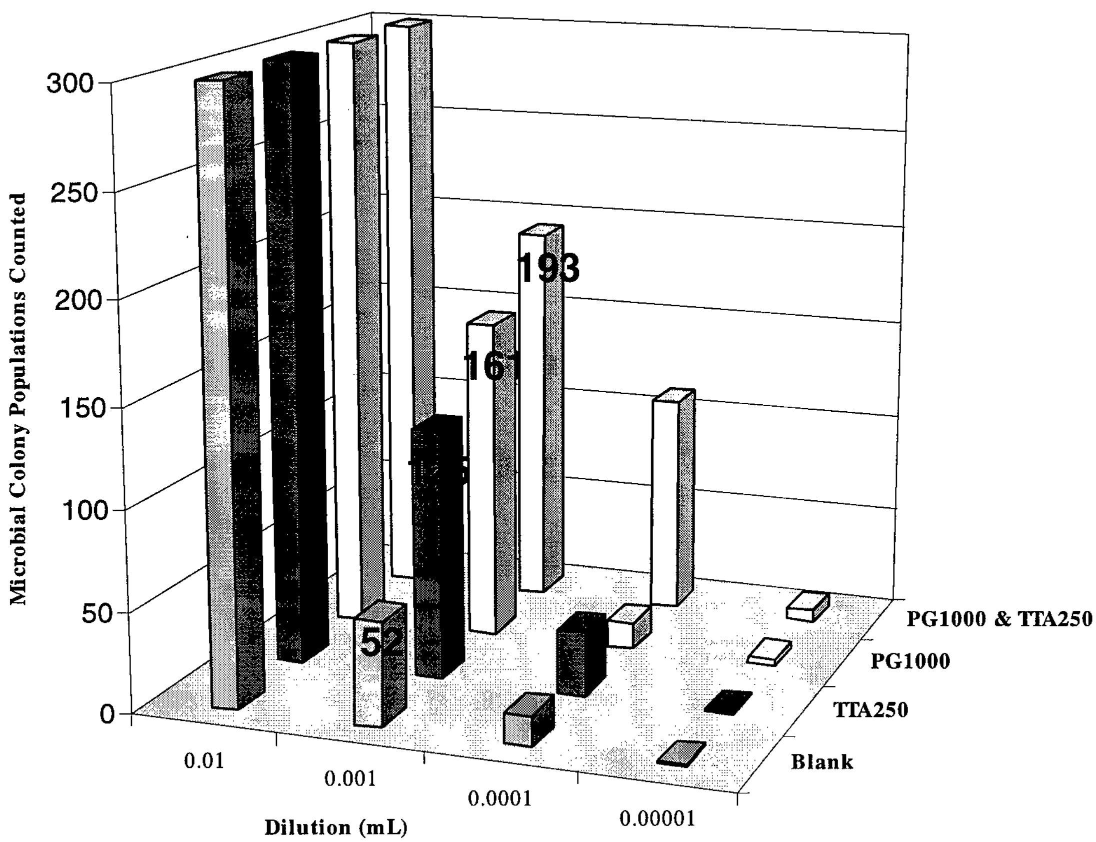
A statistical test was performed on the HPLC data to see if these additional degradation percentages (8.8% and 8.1%) were similar for the two different tolyltriazole concentrations in the presence of propylene glycol, or undeterminable due to their standard deviations. A two-sample t-test of the differences was performed using a significance level of $\alpha = 0.05$. The null hypothesis was that the additional degradation percentages were similar in value for the two different treatments of TTA. The null was accepted, and the HPLC results supported a consistent percent (8.1 – 8.8%) of additional degradation for the varied mass of TTA_{25-250} when in the presence of fixed mass of PG_{1000}.

Kellner's (1999) results of sorption/desorption of tolyltriazole with this soil showed interesting results. Using a different technique for HPLC analysis, he identified that tolyltriazole appears to strongly sorb to the organic material of the (high-clay) soil (approximately 0.7 – 1.3 mg TTA/100 gm soil). He also performed a HPLC analysis on the spent soil from this experiment. The HPLC detection areas revealed another area peak, along with the two isomers peaks of tolyltriazole. This third peak area is considered to be a reduced form of the tolyltriazole chemical, as proposed in Figure 2-2.

4.3.4 Analysis of Microbial Colony Plate Count Results

The microbial colony plate count test used spent soil from phase-one respirometry experiments. The visual results depict the influence ADF chemicals had upon microbial populations within the soil/chemical environment. Two chemical concentrations of tolyltriazole (250 mg/kg and 500 mg/kg) were tested and are shown in Figures 4-9 and Figure 4-10, respectively. Data can be found in Appendix I.

Figure 4-9
Microbial Colony Population Counts (PG_{1000} and TTA_{250})
Observation at the 48 hour point

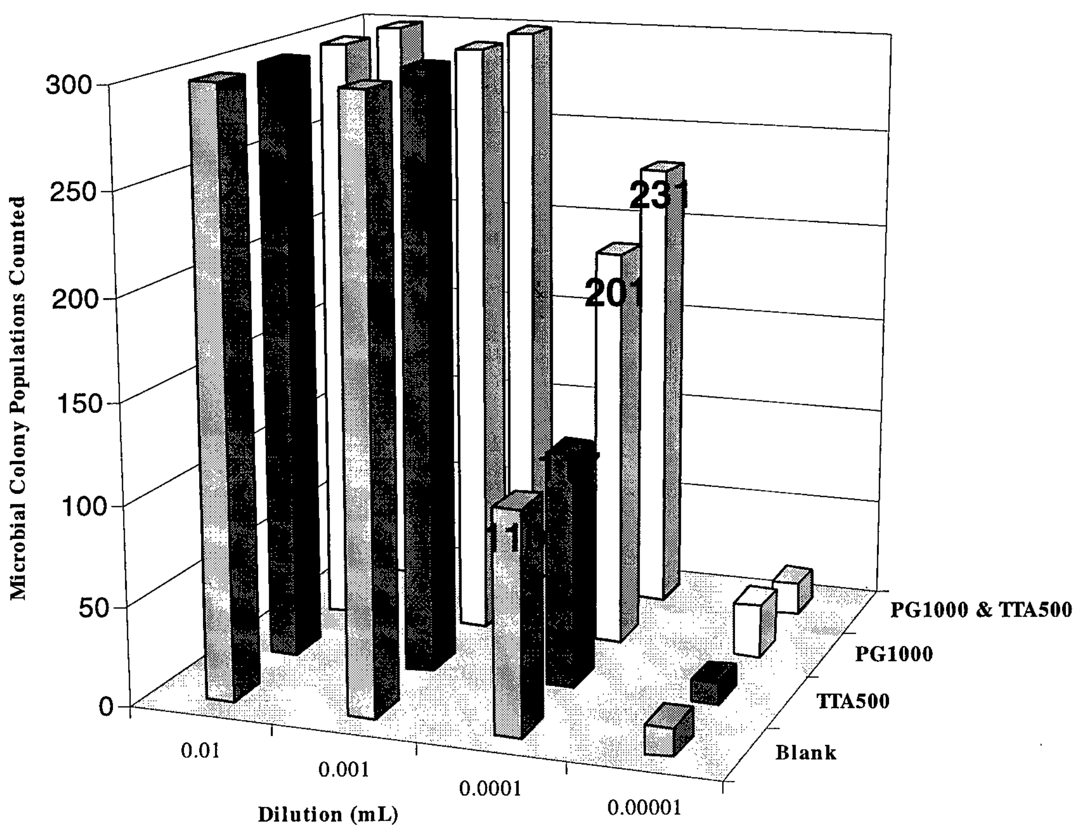


Note: Each column represents an average of three petri dishes, counted three times and averaged.

In Figure 4-9 above, the dilution range of 0.001 produced a range of 52 – 193 colonies. This range of colonies was within the acceptable range/limits of evaluation

(30 – 300) as described in *Standard Methods.* Uncontaminated soil (blank) was the base line for the population of microorganisms. The MCPC results showed that concentrations and combinations tested for PG_{1000} and TTA_{250} had no toxic effect on populations of microorganism in soil.

Figure 4-10
Microbial Population Counts (PG_{1000} and TTA_{500})
Observation at the 48 hour point



Note: Each column represents an average of three petri dishes, counted three times and averaged.

In Figure 4-10 above, the dilution range of 0.0001 produced a range of 110 – 231 colonies. This range of colonies was within the acceptable range/limits for evaluation (30 – 300) as described in *Standard Methods.* Uncontaminated soil (blank) was the base line for the population of microorganisms. The MCPC results showed that

concentrations and combinations tested for PG_{1000} and TTA_{500} had no toxic effect on populations of microorganism in soil.

Both MCPC figures indicated that these concentrations and combinations of ADF components did not affect the populations of soil microorganisms.

4.3.5 Analysis of Agar Well Diffusion Test Results

The agar well diffusion test was performed with a propylene glycol concentration of 10,000 mg/L and tolyltriazole concentrations of 5,000 – 10,000 mg/L. Individual and combined mixtures of these ADF components were applied. The tests followed the methodology section 3.7. The visual data are located in Appendix J. The results indicated no toxic effects to microbial population growth around the agar well. This indicates no toxic effects from individual and combined ADF chemical components.

4.4 Biodegradation Analysis of Respirometry Data (Phase-two)

Phase-two of this research was designed to determine if application of PG_{1000} on acclimated soil/microorganisms would produce different respiration activity. The expectation was increased biodegradation of materials, since microorganisms were acclimated to the chemicals. This would reduce lag time and increase the initial biodegradation rate of microbes.

Phase-two research also looked at the effects of residual tolyltriazole in soil. The comparison of acclimated soils (PG alone, TTA alone, and PG & TTA) new O_2 consumption rates after PG_{1000} was applied. Figure 4-11 shows various rates of O_2 consumption for combined ADF components on acclimated soil.

Figure 4-11
Rate of O_2 Consumption (μL/hr) for Propylene Glycol (1,000 mg/kg) on Uncontaminated Soil and Acclimated ADF Chemical Soils

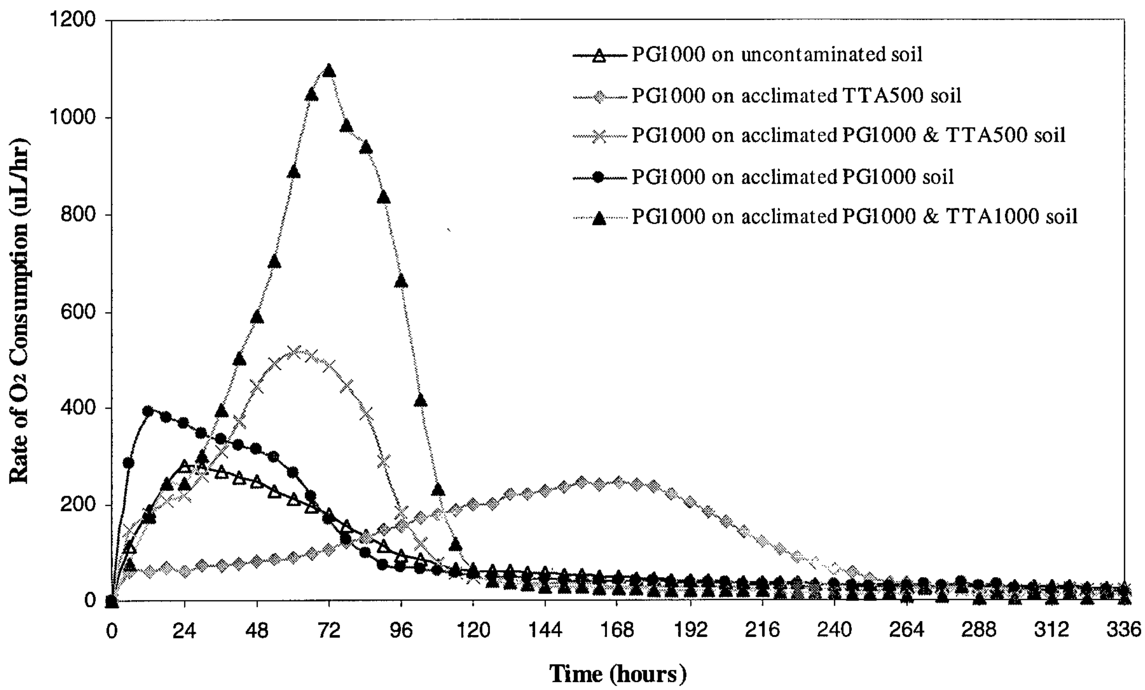
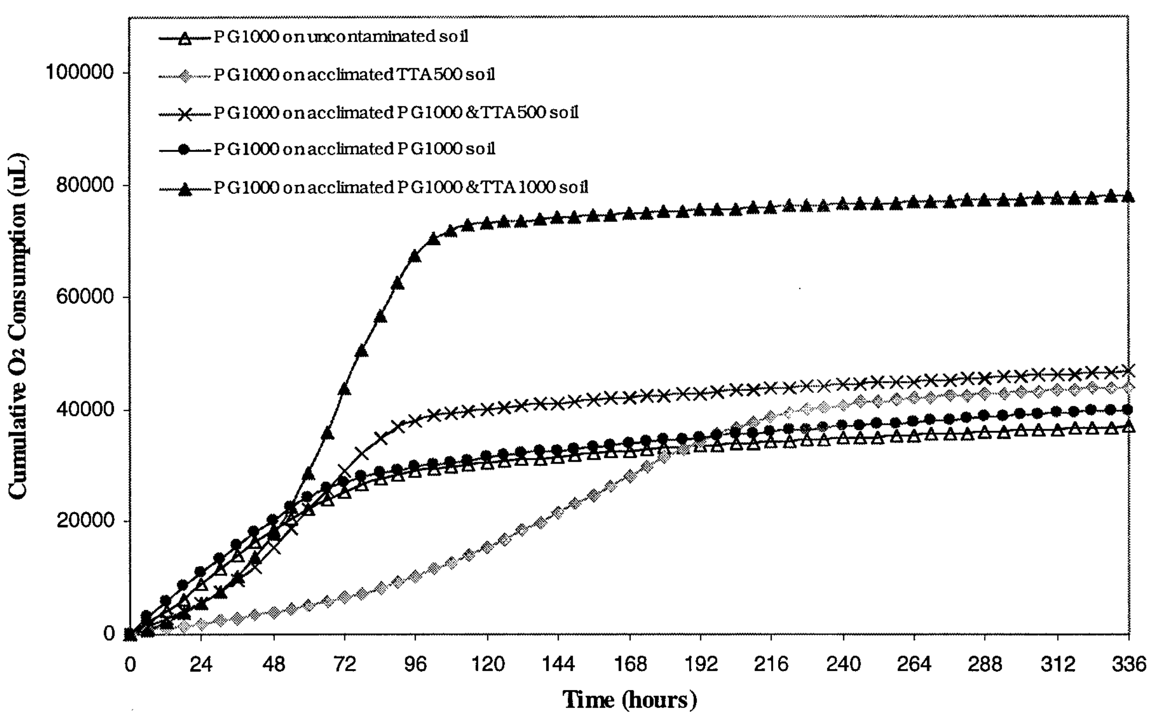


Figure 4-12
Cumulative O_2 Consumption (μL) for Propylene Glycol (1,000 mg/kg) on Uncontaminated Soil and Acclimated ADF Chemical Soils



In Figure 4-11, an unexpectedly higher cumulative O_2 consumption total (~80K μL, at 336 hr point) was noticed, and a higher rate of O_2 consumption (Figure 4-12) was observed in the acclimated PG_{1000} & TTA_{1000} soil, after PG_{1000} was applied. The reason might be residual propylene glycol slowed the rate of O_2 consumption from PG_{1000} & TTA_{1000} combination on uncontaminated soil (Figures 4-7).

There was another unexpected result for the two acclimated soils (TTA_{500} and PG_{1000} & TTA_{500}) rates of O_2 consumption (Figure 4-12). There should have been no rate difference, if the tolyltriazole residuals from the phase-one soil treatments (PG_{1000} & TTA_{500} and TTA_{500}) were equal (no loss to chemical, biological, and/or physical).

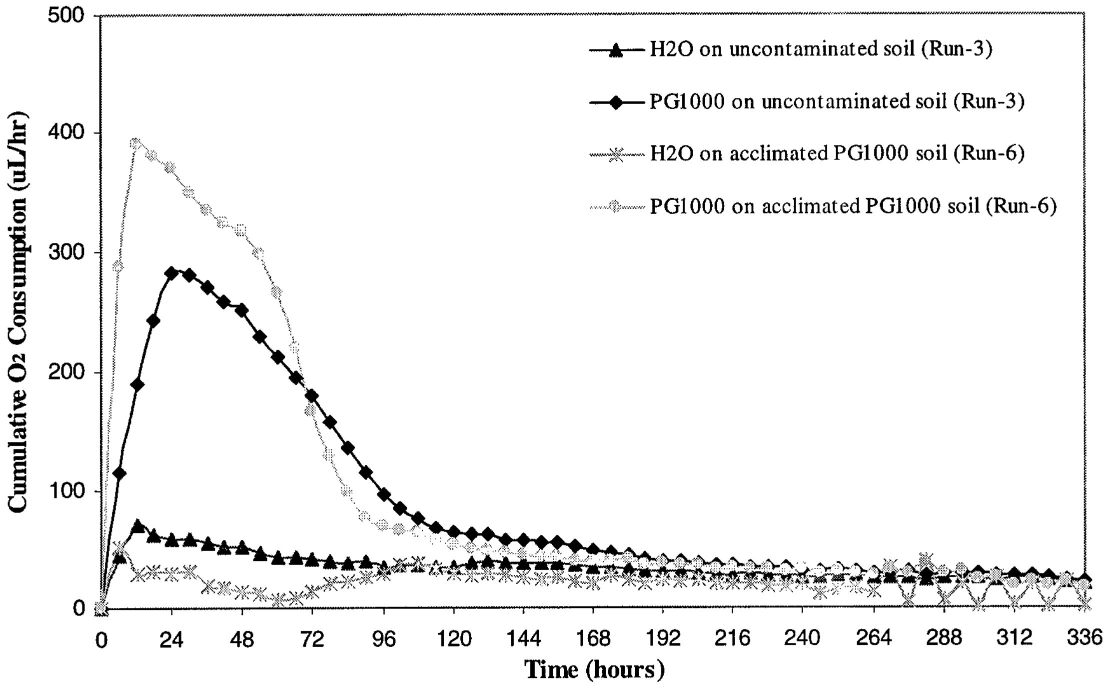
4.5 Phase-one Compared to Phase-two Initial Biodegradation Rates

Statistical testing was used to compare PG_{1000} application on uncontaminated soil (phase-one data) versus PG_{1000} re-application on PG_{1000} acclimated soil. The specific focus was to determine if there were any effects in initial O_2 consumption rates (biodegradation) from unacclimated compared to acclimated microorganism.

The statistical test used a two-tailed t-test, with a significance level of $\alpha = 0.05$. The null hypothesis was stated as: There was no difference between initial O_2 consumption rates (initial biodegradation rates) from PG_{1000} treatment on uncontaminated (phase-one) versus PG_{1000} acclimated soil (phase-two).

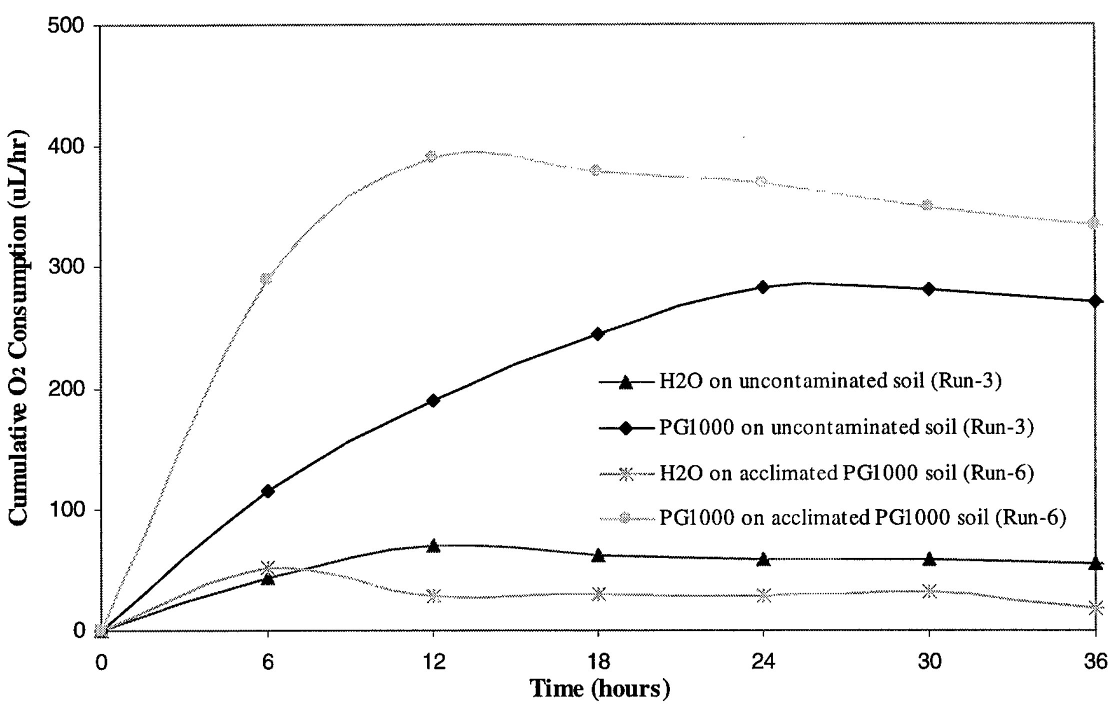
The biodegradation rates were generated from the ThOD calculations used in Appendix K. The maximum/initial biodegradation rates were visually determined by combining the applicable data from both phase-one and phase-two. Figure 4-13 combines data from Run-3 and Run-6.

Figure 4-13A
Both Phases Rate of O_2 Consumption from Respirometry Data (336 hrs of Data)



Note: Maximum/initial rates of O_2 consumption were determined with in the 24 – 36 hr time period. Figure 4-13A was enlarged to provided a more useful graph (Figure 4-13B) for visual analysis.

Figure 4-13B
Both Phases Initial Rate of O_2 Consumption from Respirometry Data (36 hrs of Data)



The first 24 hrs of cumulative O_2 consumption totals were processed using equations found in Appendix K. The calculations developed the initial biodegradation rates per mass of soil (mL/min/kg) for the two different O_2 consumption totals. The initial biodegradation rates were then statistically compared using the two-tailed t-test procedures explained in Appendix L. The results are summarized in Table 4-9 shown.

Table 4-9
Statistical Test of Acclimated versus Uncontaminated Soils
Initial Biodegradation Rates

Test Statistic $-t_{crit} \leq t^* \geq t_{crit}$	t-value t^*	t-critical t_{crit}	Reject H_o
t^* between t_{crit}, do not reject H_o	27.52	2.78	**Yes**

The null hypothesis was rejected; stating that there was a significant <u>increase</u> in initial biodegradation rates when PG_{1000} was applied on acclimated soil (with PG_{1000}) compared to the initial biodegradation rates of PG_{1000} application on uncontaminated soil.

V. Conclusions and Recommendations

5.1 Conclusions

The objective of this research was to study the effects on microbial degradation of ADF components in a (high-clay) soil environment. Previous studies have shown varied effects on microbial degradation of propylene glycol and tolyltriazole. The objective was to expand the research with varied concentrations to better understand microbial response to these chemicals.

Phase-one respirometry tests measured biodegradation effects of ADF chemicals upon uncontaminated clay soil. The ADF component propylene glycol (1,000 mg/kg) showed measurable O_2 consumption in soil compared to blank soil. The ADF component tolyltriazole (25 – 750 mg/kg) showed minimal O_2 consumption in soil compared to blank soil.

These ADF chemicals were combined to test the effects of tolyltriazole on the known O_2 consumption activity of propylene glycol in soil. Propylene glycol (1,000 mg/kg) mixed with different concentrations of tolyltriazole (25 – 1,000 mg/kg) showed varying respiration results. The rate of O_2 consumption slowed with increasing concentrations of (250 ⇒ 1,000 mg/kg) tolyltriazole with a fixed mass of (1,000 mg/kg) propylene glycol. Lower concentrations of (25 mg/kg) tolyltriazole with a fixed mass of (1,000 mg/kg) propylene glycol (similar to field conditions) showed little change in the rate of O_2 consumption. The higher concentrations of (750 – 1,000 mg/kg) tolyltriazole with a fixed mass of (1,000 mg/kg) propylene glycol had a significantly lower rate of O_2 consumption. Overall, as the (25 – 750 mg/kg) tolyltriazole increased with a fixed (1,000

mg/kg) propylene glycol, the O_2 consumption totals increased.

ThOD calculations for microbial degradation of these two components supported the idea of tolyltriazole's biodegredation with propylene glycol. In other words, as tolyltriazole increased in concentrations, a proportional (ThOD calculations = equation for microbial breakdown of chemicals) amount of O_2 consumption occurred. This supports the biodegradation/breakdown of tolyltriazole with propylene glycol.

The HPLC data could not demonstrate the biodegradation potential of tolyltriazole in soil, due to numerous degradation pathways (chemical, physical, and/or biotic). However, the potential for a biodegradation pathway was associated with the lower concentrations of (25 – 250 mg/kg) tolyltriazole when in the presence of (1,000 mg/kg) propylene glycol. HPLC results showed additional degradation (8.1 – 8.8%) of tolyltriazole mass occurred when in the presence of a fixed amount of propylene glycol. This supported the increased O_2 consumption totals as the mass of tolyltriazole increased when in the presence of a fixed mass of propylene glycol.

In conclusion of phase-one results, the respirometry data would imply that (1,000 mg/kg) propylene glycol biodegrades alone in soil, while little to no biodegradation occurs for (25 – 750 mg/kg) tolyltriazole alone in soil. Respirometry and HPLC data implies some potential biodegradation of (25 – 500 mg/kg) tolyltriazole mass in the presence of (1,000 mg/kg) propylene glycol.

The MCPC test revealed that the populations of microbes, acclimated in soil contaminated with ADF components, appeared to stay consistent or higher than microbial populations in uncontaminated soil. The AWDT reveled that microbes would grow upon solutions of ADF components (TTA and/or PG) without inhibition. Both of the toxicity

tests showed no adverse effects upon microorganisms in soil from tolyltriazole and propylene glycol chemicals.

Phase-two of this study evaluated biodegradation when propylene glycol was re-applied to acclimated soil from the phase-one study. Focus was on the comparison of (1,000 mg/kg) propylene glycol initial rate of biodegradation (O_2 consumption) on uncontaminated soil and acclimated soil (with propylene glycol only). Table 5-1 summarizes the initial biodegradation rates calculated from the respirometry data.

Table 5-1
Initial Biodegradation Rates for Propylene Glycol (1,000 mg/kg) Application on Propylene Glycol Acclimated Soil and Uncontaminated Clay Soil

Propylene Glycol (1,000 mg/kg) Application	
Uncontaminated Soil	Acclimated Soil
Biodegradation Rate (mL/day/kg soil)	Biodegradation Rate (mL/day/kg soil)
107.41	148.81

Statistical tests supported the idea that when propylene glycol (1,000 mg/kg) was applied to both acclimated and uncontaminated soil, the initial biodegradation rate of acclimated soil was significantly faster than the rate for uncontaminated soil.

5.2 Improvements

5.2.1 Use of HPLC with Indirect UV Detection

The use of HPLC methods with indirect UV detection has been established using derivatization [Massaccesi, 1992]. This could be applied to residual propylene glycol in the soil.

5.2.2 Modifying the HPLC with Refractive Index Detection

The modification of the HPLC with refractive index detection equipment is another approach for propylene glycol detection in the aqueous phase. The protocols and detection limits are established (Nitschke *et al.*, 1994) for this refractive index detection. This could provide a mass accounting of propylene glycol after respirometry research.

5.2.3 Gas Chromatography with Flame Ionization Detection

The use of Gas Chromatography with Flame Ionization Detection (GC/FID) has been established by methods used in Kaplan *et al.* (1982) research on glycol. These methods of GC/FID could be applied to the residual propylene glycol in soil.

5.2.4 Modifying the Respirometer

The addition of ammonia and methane detection equipment to the respirometer would provide possible investigations in anaerobic conditions. This is one of the proposed pathways for the biodegradation of tolyltriazole.

5.3 Follow-on Research

5.3.1 Investigating other components in ADFs

There are several other additives within the ADFs. The biodegradation potential of one or more of these additives with propylene glycol would reveal other interaction effects on biodegradation potential.

5.3.2 Multiple Recontamination of ADF Components on Soil

A possible area of focus would be multiple applications of ADF components on soil. Developing an overall biodegradation rate trend from the various recontamination phases could be the focus question. The research could develop a long-term trend of increased/steady-state/decreased biodegradation rates for the ADF components. Then development and optimization of ADF application cycles on soil could be approached. Some examples might be the following:

1. (PG_{1000} & TTA_{10}) then (PG_{1000} & TTA_{10}) then (de-ionized water) → repeat cycle, or
2. (PG_{1000} & TTA_{10}) then (de-ionized water) then (PG_{1000} & TTA_{10}) → repeat cycle, or
3. (PG_{1000} & TTA_{10}) then (PG_{1000}) then (PG_{1000} & TTA_{10}) → repeat cycle

5.3.3 Field Tests of ADF Component Biodegradation

Field testing ADF component degradation (bio and chemical) in an *in-situ* environment. Through establishment of a test area, application of different concentration and combinations of ADF components could be studied. HPLC or GC/FID analysis of residual concentrations might be applied to determine field versus laboratory results.

Appendix A:
Independent Soil Analysis

AFIT/ENV/Charles A Bleckmann
2950 P Street
Wright-Patterson AFB OH 45440

Colorado State University
Soil, Water and Plant Testing Laboratory
Natural & Environmental Sciences Bldg - A319
Fort Collins, CO 80523

(970) 491-5061 FAX: 491-2930

DATE RECEIVED: 12-14-1998
DATE PARTIAL REPORTED: 01-22-1999
DATE REPORTED: 02-16-1999

BILLING:

RESEARCH SOIL ANALYSIS

8-Dec-98

Lab #	Sample ID #	paste		Lime Estimate	%	AB-DTPA Extract ppm						
		pH	EC mmhos/cm		OM	NO_3-N	P	K	Zn	Fe	Mn	Cu
R3392	Afit # 1	7.8	1.2	Medium	2.7	3.3	5.3	91.7	2.44	50.0	2.89	2.93
R3393	Afit # 2	7.7	0.9	Medium	2.9	6.2	4.9	92.0	2.63	51.7	2.76	3.15
R3394	Afit # 3	7.8	0.8	Medium	3.0	6.2	5.6	99.2	2.70	50.2	2.63	2.89

Lab #	Sample ID #	%			Texture	%
		Sand	Silt	Clay		TOC
R3392	Afit # 1	48	36	16	Loam	1.61
R3393	Afit # 2	48	36	16	Loam	1.82
R3394	Afit # 3	49	35	16	Loam	1.78

Appendix B: Calculations of Field Capacity and Solution Concentrations for Experiments

Field capacity test of (high-clay) soil (September 18, 1998)

$M_s := 97.8$ gm Mass of soil in situ condition

$M_w := 18.4$ gm Mass of water absorbed into soil to achieve 100% FC.
(24 hrs at saturation, 2 hrs drainage)

$FC := \frac{M_w}{M_s}$ $FC = 0.19$

Amount of soil with water that totals 50 grams in microcosm to achieve ~ 60% of FC of the soil

$M_{soil} := 45$ gm Mass of soil (in situ) to achieve ~60% of FC to equal 50 grams total mass after addition of water

$FC = 0.188$ Field capacity of water within soil to achieve 100%

$FC\% := .60$ Percentage (~60%) range of field capacity ratio determined above

$M_{H2O} := (M_{soil}) \cdot (FC) \cdot (FC\%)$ $M_{H2O} = 5.1 \cdot gm$

$M_{H2O} := 5.0 \cdot gm$ <--- This is the amount of liquid added to 45 grams soil to achieve ~60% FC. Note It was rounded to 5 gm H2O to make inoculation easier within the microcosms.

$M_{sw} := 50$ gm <----- Mass of ~60% FC soil (Mass of soil and water together)

The addition of 5.0 grams of H20 solution (PG only, TTA only, or PG & TTA) requires a specific concentration to achieve the designed application desired in parts per million (ppm) that is equal to mg contaminant/kg soil.

Example Calculations:

Experimental treatment of PG used in all runs ------> $PG1000_{ppm} := 1000 \frac{mg}{kg}$

Formula: $\frac{1000 \text{ mg PG}}{1 \text{ kg soil}} = \frac{X \text{ mg PG}}{50 \text{ gm soil}}$

Formula: $X \text{ mg PG} = \frac{1000 \text{ mg PG}}{1 \text{ kg soil}} * (50 \text{ gm soil})$

Mathcad Formula: $PG1000_{mass} := (PG1000_{ppm}) \cdot (M_{sw})$

$PG1000_{mass} = 50 \cdot mg$ <--- Mass of PG required for 50 grams of ~60% FC soil = 1,000 mg/kg

Experimental treatment of TTA25 -------> $TTA_{25ppm} := 25 \frac{mg}{kg}$
(experimental Run-1)

Formula: $X \text{ mg TTA} = \frac{25 \text{ mg TTA}}{1 \text{ kg soil}} * (50 \text{ gm soil})$

Mathcad Formula: $TTA25_{mass} := (TTA_{25ppm}) \cdot (M_{sw})$

$TTA25_{mass} = 1.25 \cdot mg$ <-- Mass of TTA for 50 grams of ~60% FC soil = 25 mg/kg

Example concentration are calculated below for the solutions used in treatment of the soil (PG only, TTA only). The following formulas were used.

Required concentration for PG1000 (50 mg PG / 50 gm soil) requires 5 mL injection into soil.

Mathcad Formula: $PG1000_{mass} := (PG1000_{ppm}) \cdot (M_{sw})$

Mathcad Formula: $PG1000_{conc} := \left(\frac{PG1000_{mass}}{M_{H2O}}\right) \cdot \left(\frac{1 \text{ gm}}{1 \text{ mL}}\right) \cdot \left(1000 \frac{mL}{L}\right)$

$PG1000_{conc} = 10000 \cdot \frac{mg}{L}$ <----- Concentration required

Required concentration for TTA25 (1.25 mg PG / 50 gm soil) requires 5 mL injection into soil.

Formula: $\text{TTA conc} = \frac{1.25 \text{ mg TTA}}{5.0 \text{ mg H2O}} * \frac{1 \text{ gm H2O}}{1 \text{ mL H2O}} * \frac{1000 \text{ mL}}{1 \text{ L}}$

Mathcad Formula: $TTA25_{conc} := \left(\frac{TTA25_{mass}}{M_{H2O}}\right) \cdot \left(\frac{1 \text{ gm}}{1 \text{ mL}}\right) \cdot \left(1000 \frac{mL}{L}\right)$

$TTA25_{conc} = 250 \cdot \frac{mg}{L}$ <----- Concentration required

Appendix C: Preparation of Solutions for Inoculation of Microcosms

Materials used:

Chemicals used:

Propylene Glycol (aqueous), Laboratory Grade (Mallinckrodt OR, 1925: 1,2-Propanediol)

Tolyltriazole (solid), Manufacturer Grade (COBRATEC TT-100, Tolyltriazole, Sample 4239701)

Equipment used:

$Flask_{200mL} := 200\ mL$

$Flask_{500mL} := 500\ mL$

Concentrations required for experiments:

$PG1000_{conc} := 10000 \cdot \frac{mg}{L}$

$TTA25_{conc} := 250 \cdot \frac{mg}{L}$

$TTA250_{conc} := 2500 \cdot \frac{mg}{L}$

$TTA500_{conc} := 5000 \cdot \frac{mg}{L}$

$TTA750_{conc} := 7500 \cdot \frac{mg}{L}$

$TTA1000_{conc} := 10000 \cdot \frac{mg}{L}$

Example calculations for solution preparation of PG or TTA within a flask volume:

Formula: X mg material = Material conc (mg/L) * Flask volume (mL) $\frac{*1\ L}{1000\ mL}$

PG solution at 10,000 mg/L

$$PG_{mg} := \left(PG1000_{conc} \cdot Flask_{500mL}\right) \frac{L}{1000\ mL}$$

$PG_{mg} = 5 \cdot gm$ <-- Amount of PG (liquid) mixed with 500 mL of the de-ionized water

TTA solution at 250 mg/L

$$TTA25_{mass} := \left(TTA25_{conc} \cdot Flask_{200mL}\right) \frac{L}{1000\ mL}$$

$TTA25_{mass} = 0.05 \cdot gm$ <-- Amount of TTA (solid) mixed with 200 mL of solution (de-ionized water or PG 10,000 mg/L solution)

Appendix D: Calculations for HPLC Calibration Curve for Tolyltriazole

ORIGIN≡1

$$\text{Known\_Concentration\_Level}_{TTA} := \begin{bmatrix} 1 \\ 5 \\ 10 \\ 50 \\ 100 \\ 1000 \end{bmatrix}$$

The concentration of 1,000 mg/L TTA was developed first, then diluted to prepare the weaker concentrations.

NOTE: All calibration solutions are based with HPLC grade methanol. Since the extraction process of TTA from the spent soil uses a large proportion of methanol.

$$X := \text{Known\_Concentration\_Level}_{TTA}$$

Table D-1
HPLC Calibration Curve Data for Tolyltriazole

HPLC Calibration Curve Data, Tolyltriazole								
		Run 1 (23 Sep 98)		Run 2 (24 Sep 98)				
Concentration		(mAu^2)	Average	(mAu^2)	Average		Run Average	Run Std Dev
1000 mg/L	Sample 1	9204.9063		8981.4375				
	Sample 2	9192.7627		8919.0928				
	Sample 3	9106.6846	9168.1179	8930.8477	8943.7927	--->	9055.9553	43.3375
100 mg/L	Sample 1	1148.9069		1120.7009				
	Sample 2	1146.3660		1104.5593				
	Sample 3	1130.3009	1141.8579	1099.9812	1108.4138	--->	1125.1359	10.4867
50 mg/L	Sample 1	536.4797		513.9089				
	Sample 2	525.3796		512.1735				
	Sample 3	523.9478	528.6024	521.6556	515.9127	--->	522.2575	5.9540
10 mg/L	Sample 1	112.3766		109.4433				
	Sample 2	115.9473		111.1758				
	Sample 3	113.1561	113.8267	111.0857	110.5683	--->	112.1975	1.4264
5 mg/L	Sample 1	58.1064		56.4408				
	Sample 2	58.6636		56.2115				
	Sample 3	57.2977	58.0226	55.9262	56.1928	--->	57.1077	0.4723
1 mg/L	Sample 1	13.1479		13.0238				
	Sample 2	13.3671		13.0937				
	Sample 3	13.1933	13.2361	13.1003	13.0726	--->	13.1544	0.0790

$$\text{Observed\_Detection\_Areas}_{TTA} := \begin{bmatrix} 13.1544 \\ 57.1077 \\ 112.1975 \\ 522.2575 \\ 1125.1359 \\ 9055.9553 \end{bmatrix}$$

The detection area for each standard was performed three times and averaged to produce the data listed in "$\text{Observed\_Detection\_Areas}_{TTA}$".

$Y := \text{Observed\_Detection\_Areas}_{TTA}$

Calculation for the linear best fit line:

$m := \text{slope}(X, Y)$ $m = 9.01$ <--- Calculation of slope

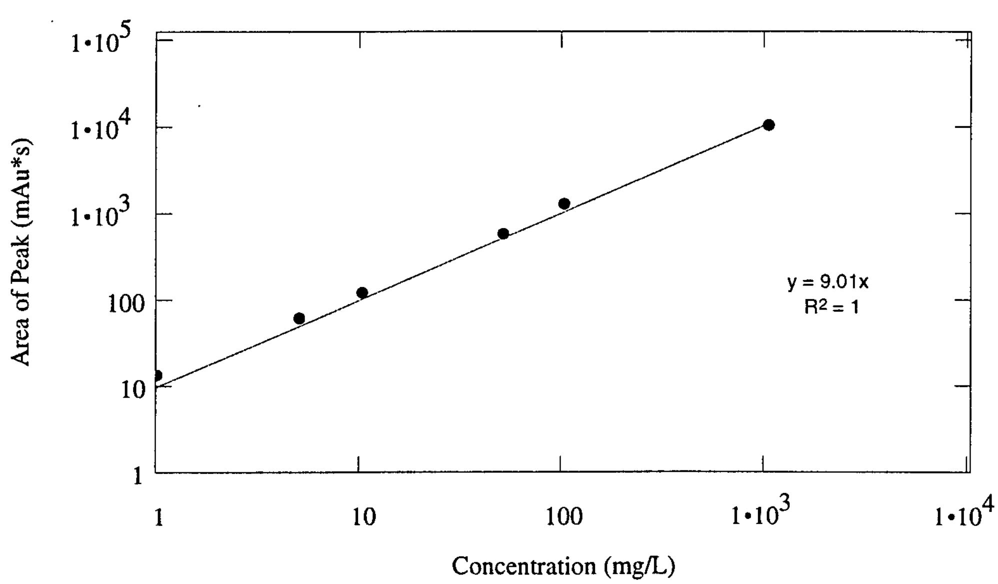
$r := \text{corr}(X, Y)$ $r = 0.9997$ <--- Calculation of the correlation between concentration and area peaks

MathCad 7.0 uses Pearson correlation coefficient

$y(x) := m \cdot x$ <--- Equation of the linear line

$y(\log x) := m \cdot \log x$ <--- Log scale is applied to enable a more usable graph, thus lower concentration levels can be calculated from the integrated areas from HPLC detection

Figure D-1
Calibration Curve for Tolyltriazole



Level of Detection (LOD) is provided by the formula:

$LOD = 3*s_{Total}$ <--- $s_{Total}^2 = s_{Background}^2 + s_{Observed}^2$

$\sigma_{Background} := 0$ <--- Noise is eliminated from integration of areas in HP Chem. Station software

$$Std\_Dev := \begin{bmatrix} 43.3375 \\ 10.4867 \\ 5.9540 \\ 1.4264 \\ .4723 \\ .0790 \end{bmatrix}$$

$\sigma_{Observed} := mean(Std\_Dev)$

$\sigma_{Observed} = 10.293$

$$\sigma_{Total} := \sqrt{\sigma_{Background}^2 + \sigma_{Observed}^2}$$

$LOD_{areas} := 3 \cdot \sigma_{Total}$

$LOD_{areas} = 30.878$ <--- mAu*s

$$LOD_{conc} := \frac{LOD_{areas}}{9.01}$$

$LOD_{conc} = 3.427$ <--- mg/L

Appendix E: Respirometry Data

All respirometry experiments were conducted in accordance with the methodology section. Table E-1 is a detailed layout of all treatments for the experimental runs.

Table E-1
Layout of All Respirometry Treatments/Experiments

Run 1

Bottle	1	2	3	4	5
Treatment	TTA_{25}	TTA_{25}	TTA_{25}	TTA_{25}	TTA_{25}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	6	7	8	9	10
Treatment	Empty	Empty	PG_{1000} & TTA_{25}	PG_{1000} & TTA_{25}	PG_{1000} & TTA_{25}
Soil Type	Bottle	Bottle	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	11	12	13	14	15
Treatment	PG_{1000} & TTA_{25}	PG_{1000} & TTA_{25}	Blank/H_20	Blank/H_20	Blank/H_20
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	16	17	18	19	20
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Run 2

Bottle	1	2	3	4	5
Treatment	TTA_{250}	TTA_{250}	TTA_{250}	TTA_{250}	TTA_{250}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	6	7	8	9	10
Treatment	Empty	Empty	PG_{1000} & TTA_{250}	PG_{1000} & TTA_{250}	PG_{1000} & TTA_{250}
Soil Type	Bottle	Bottle	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	11	12	13	14	15
Treatment	PG_{1000} & TTA_{250}	PG_{1000} & TTA_{250}	Blank/H_20	Blank/H_20	Blank/H_20
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	16	17	18	19	20
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Run 3

Bottle	1	2	3	4	5
Treatment	TTA_{500}	TTA_{500}	TTA_{500}	TTA_{500}	TTA_{500}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	6	7	8	9	10
Treatment	Empty	Empty	PG_{1000} & TTA_{500}	PG_{1000} & TTA_{500}	PG_{1000} & TTA_{500}
Soil Type	Bottle	Bottle	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	11	12	13	14	15
Treatment	PG_{1000} & TTA_{500}	PG_{1000} & TTA_{500}	Blank/H_20	Blank/H_20	Blank/H_20
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	16	17	18	19	20
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Run 4

Bottle	1	2	3	4	5
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Run-3, Bottle 1 TTA_{500}	Run-3, Bottle 2 TTA_{500}	Run-3, Bottle 3 TTA_{500}	Run-3, Bottle 4 TTA_{500}	Run-3, Bottle 5 TTA_{500}

Bottle	6	7	8	9	10
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Blank Uncontaminated	Blank Uncontaminated	Run-3, Bottle 8 PG_{1000} & TTA_{500}	Run-3, Bottle 9 PG_{1000} & TTA_{500}	Run-3, Bottle 10 PG_{1000} & TTA_{500}

Bottle	11	12	13	14	15
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Run-3, Bottle 11 PG_{1000} & TTA_{500}	Run-3, Bottle 12 PG_{1000} & TTA_{500}	Blank Uncontaminated	Blank Uncontaminated	Blank Uncontaminated

Bottle	16	17	18	19	20
Treatment	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Run-3, Bottle 16 PG_{1000}	Run-3, Bottle 17 PG_{1000}	Run-3, Bottle 18 PG_{1000}	Run-3, Bottle 19 PG_{1000}	Run-3, Bottle 20 PG_{1000}

Run 5

Bottle	1	2	3	4	5
Treatment	TTA_{750}	TTA_{750}	TTA_{750}	TTA_{750}	TTA_{750}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	6	7	8	9	10
Treatment	PG_{1000}	PG_{1000}	PG_{1000} & TTA_{750}	PG_{1000} & TTA_{750}	PG_{1000} & TTA_{750}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	11	12	13	14	15
Treatment	PG_{1000} & TTA_{750}	PG_{1000} & TTA_{750}	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Bottle	16	17	18	19	20
Treatment	PG_{1000} & TTA_{1000}	PG_{1000} & TTA_{1000}	PG_{1000} & TTA_{1000}	PG_{1000} & TTA_{1000}	PG_{1000} & TTA_{1000}
Soil Type	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated	Uncontaminated

Run 6

Bottle	1	2	3	4	5
Treatment	Blank/H_20	Blank/H_20	Blank/H_20	Blank/H_20	Blank/H_20
Soil Type	Mix Run-4 & Run-5 Bottle 6, PG_{1000}	Mix Run-4 & Run-5 Bottle 7, PG_{1000}	Mix Run-4 & Run-5 Bottle 13, PG_{1000}	Mix Run-4 & Run-5 Bottle 14, PG_{1000}	Mix Run-4 & Run-5 Bottle 15, PG_{1000}

Bottle	6	7	8	9	10
Treatment	Blank/H_20	Blank/H_20	PG_{1000}	PG_{1000}	PG_{1000}
Soil Type	Uncontaminated Soil	Uncontaminated Soil	Run-5, Bottle 16 PG_{1000} & TTA_{1000}	Run-5, Bottle 17 PG_{1000} & TTA_{1000}	Run-5, Bottle 18 PG_{1000} & TTA_{1000}

Bottle	11	12	13	14	15
Treatment	PG_{1000}	PG_{1000}	Blank/H_20	Blank/H_20	Blank/H_20
Soil Type	Run-5, Bottle 19 PG_{1000} & TTA_{1000}	Run-5, Bottle 20 PG_{1000} & TTA_{1000}	Uncontaminated Soil	Uncontaminated Soil	Uncontaminated Soil

List of Figures

Figure E-1 Averaged Cumulative O_2 Consumption (uL), Experimental Run-1

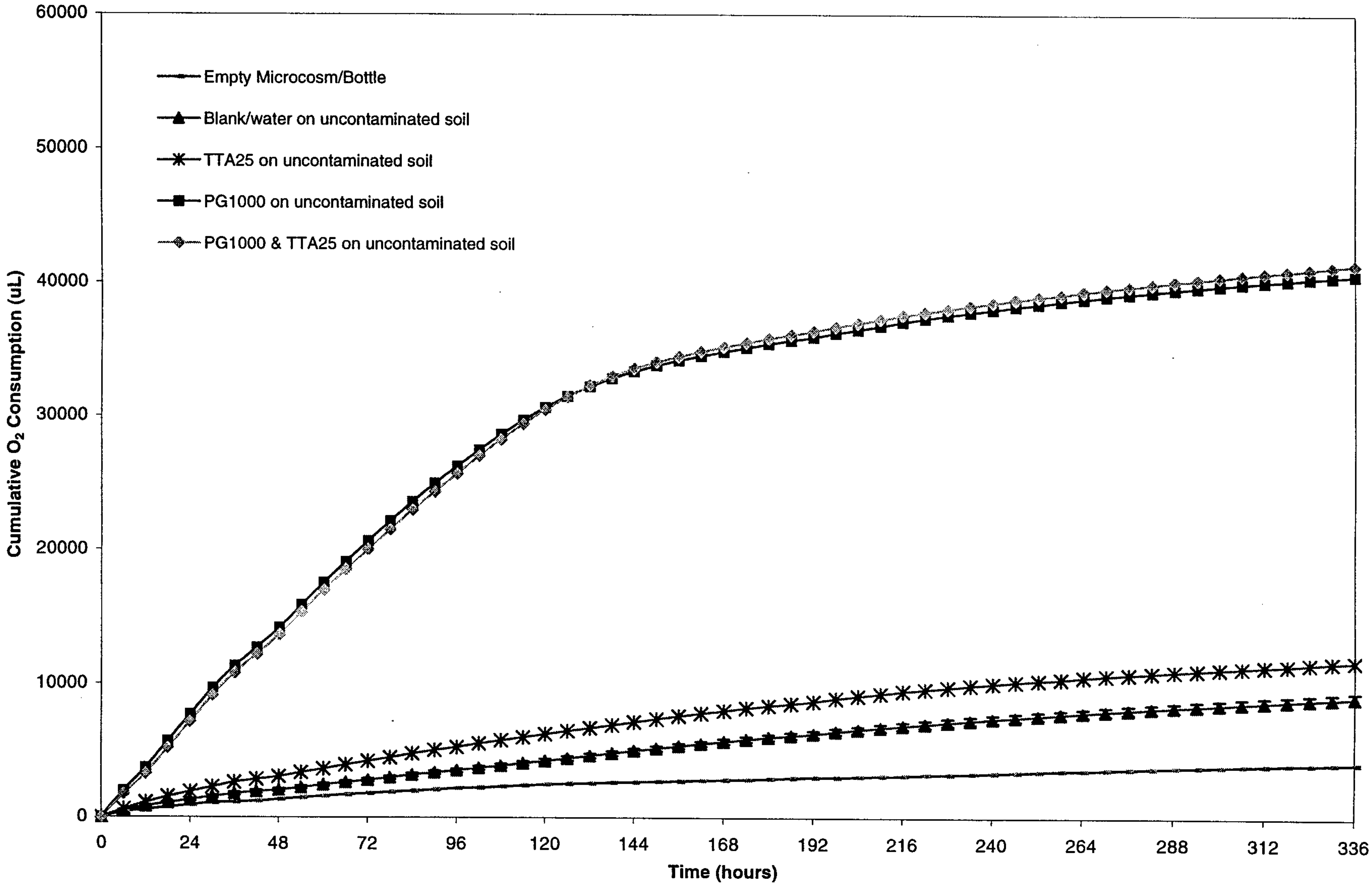


Figure E-2 Averaged Cumulative CO_2 Production (uL), Experimental Run-1

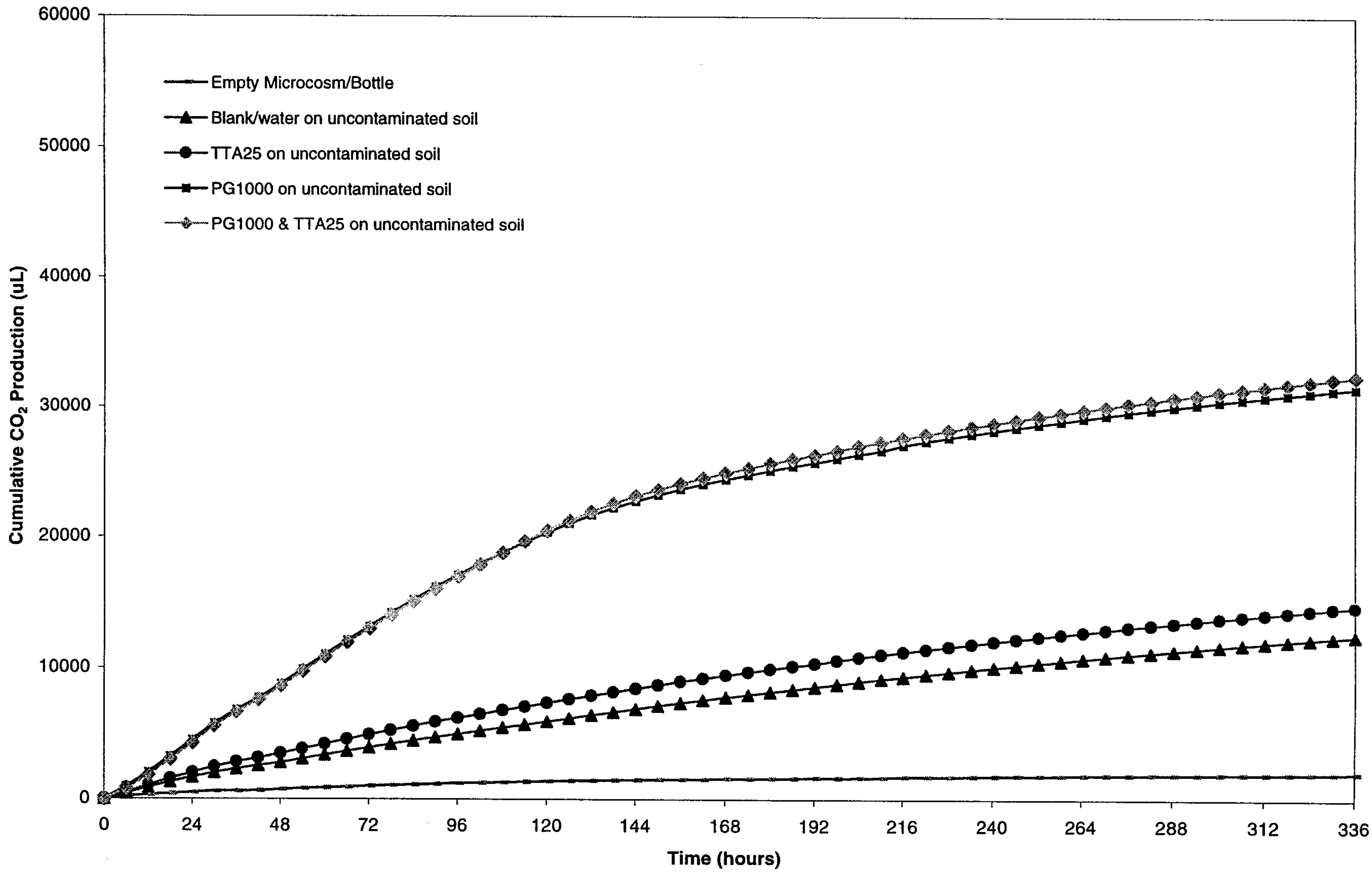


Figure E-3 Averaged O_2/CO_2 Ratio (uL/uL), Experimental Run-1

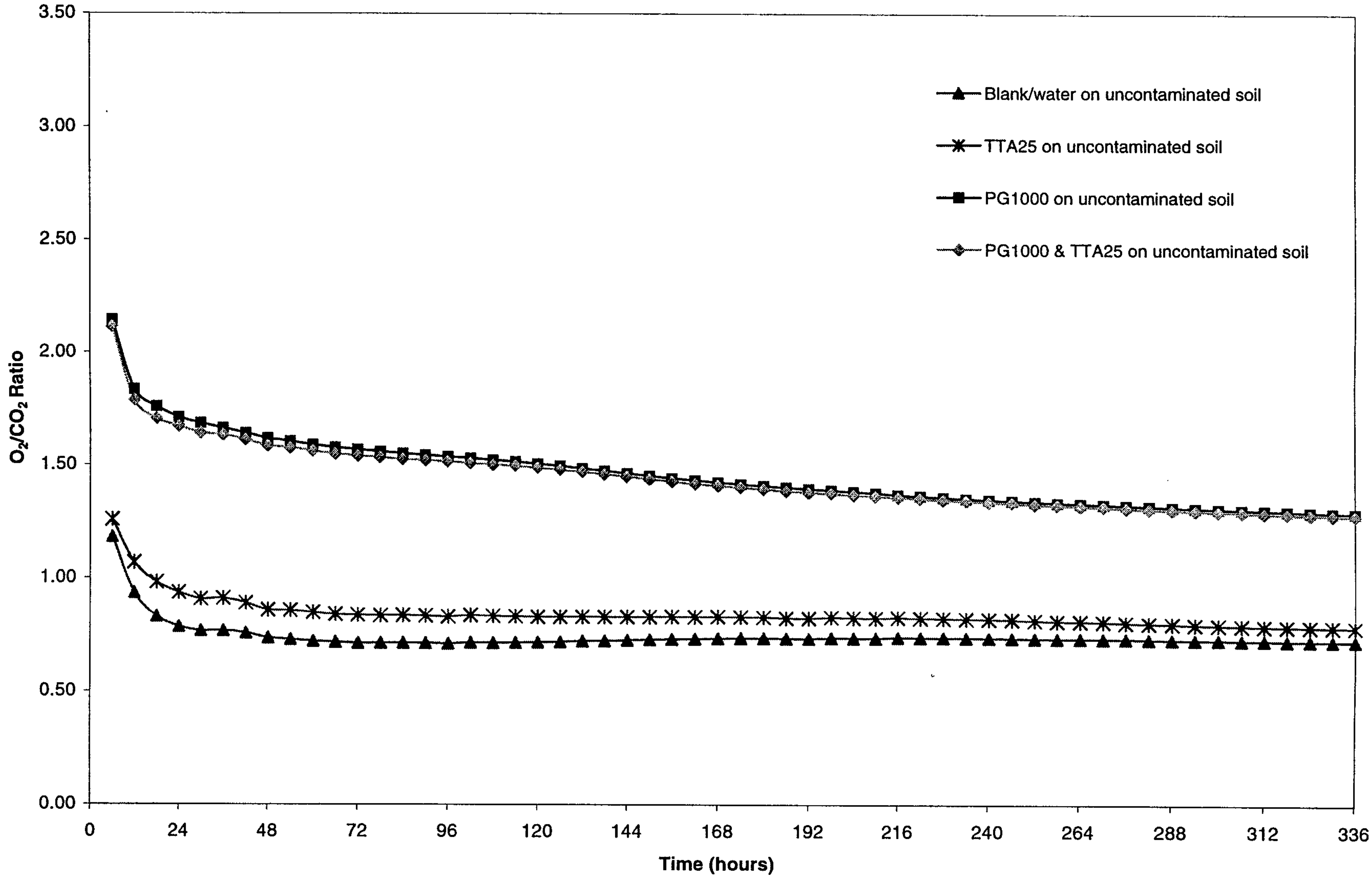


Figure E-4 Averaged Rate of O_2 Consumption (uL/hr), Experimental Run-1

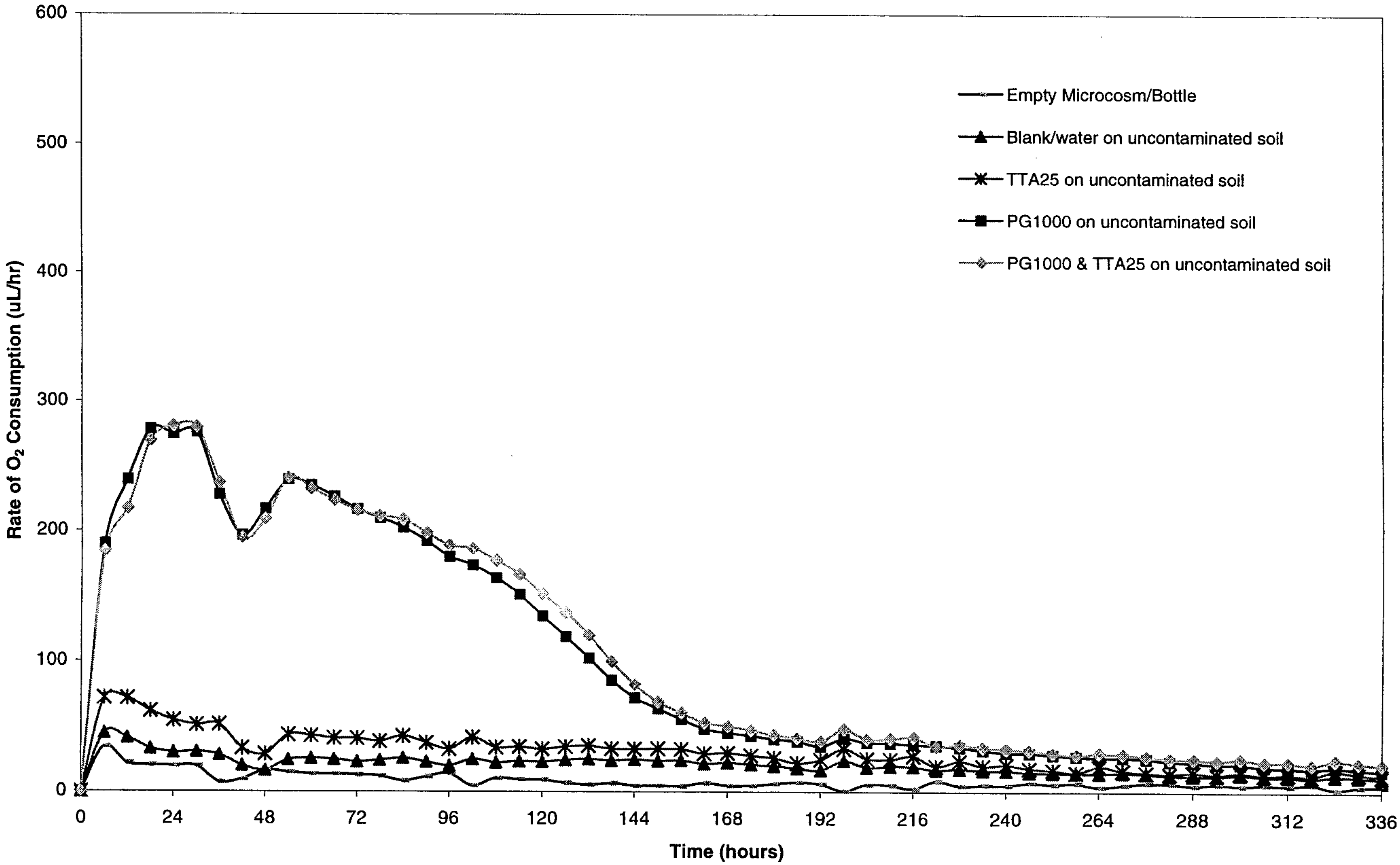


Figure E-5 Averaged Rate of CO_2 Production (uL/hr), Experimental Run-1

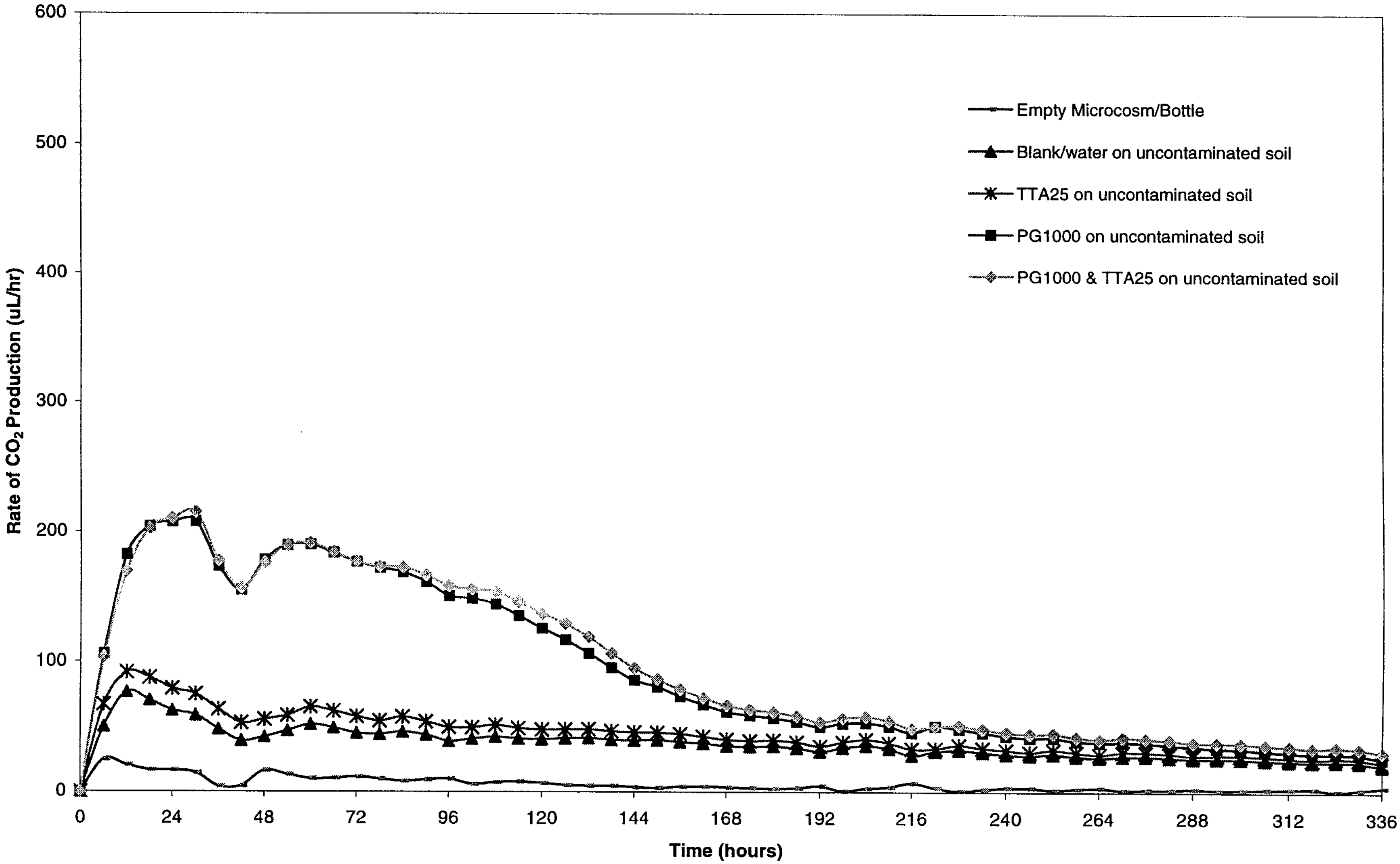


Figure E-6 Averaged Cumulative O_2 Consumption (uL), Experimental Run-2

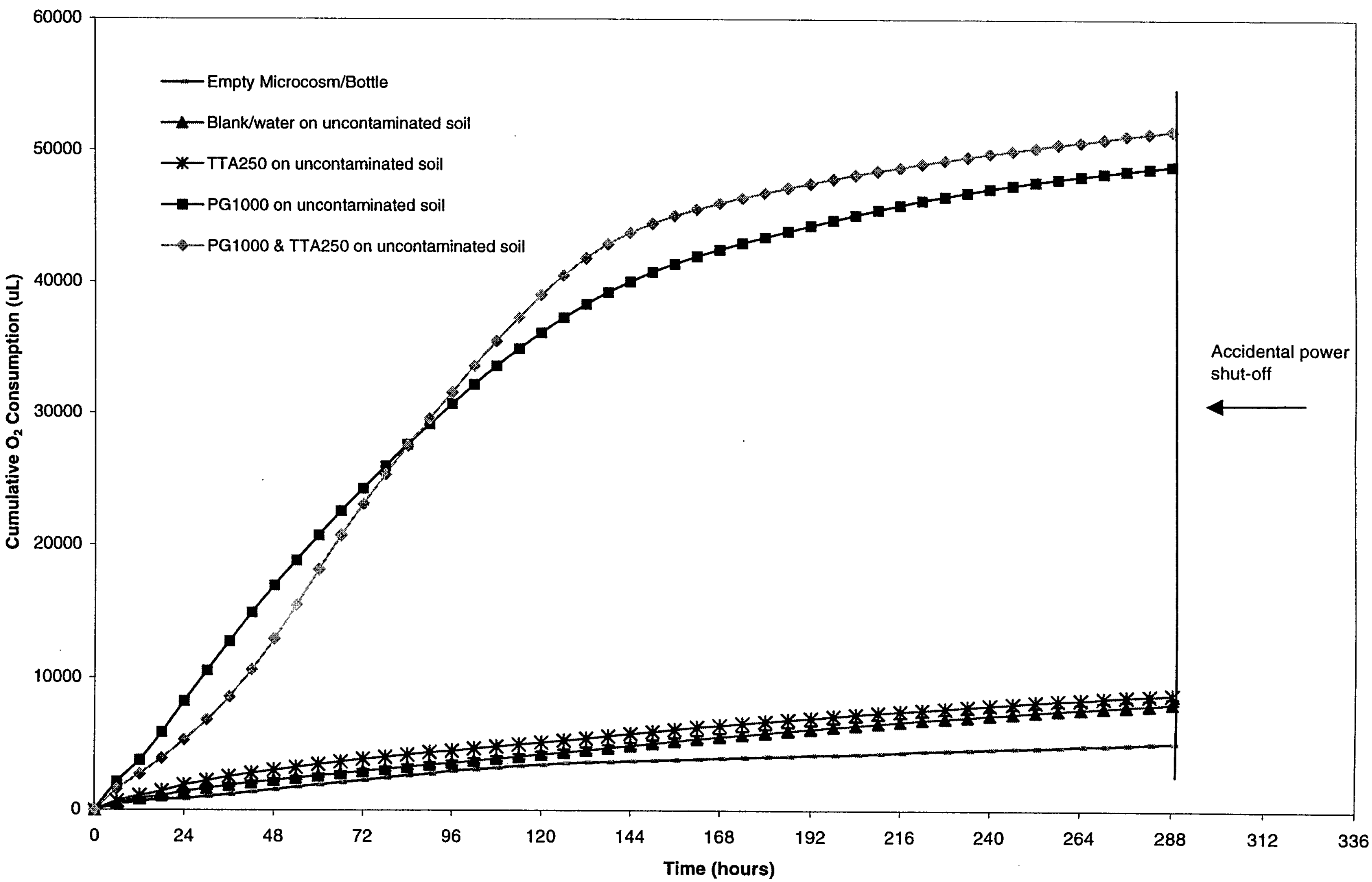


Figure E-7 Averaged Rate of O_2 Consumption (uL/hr), Experimental Run-2

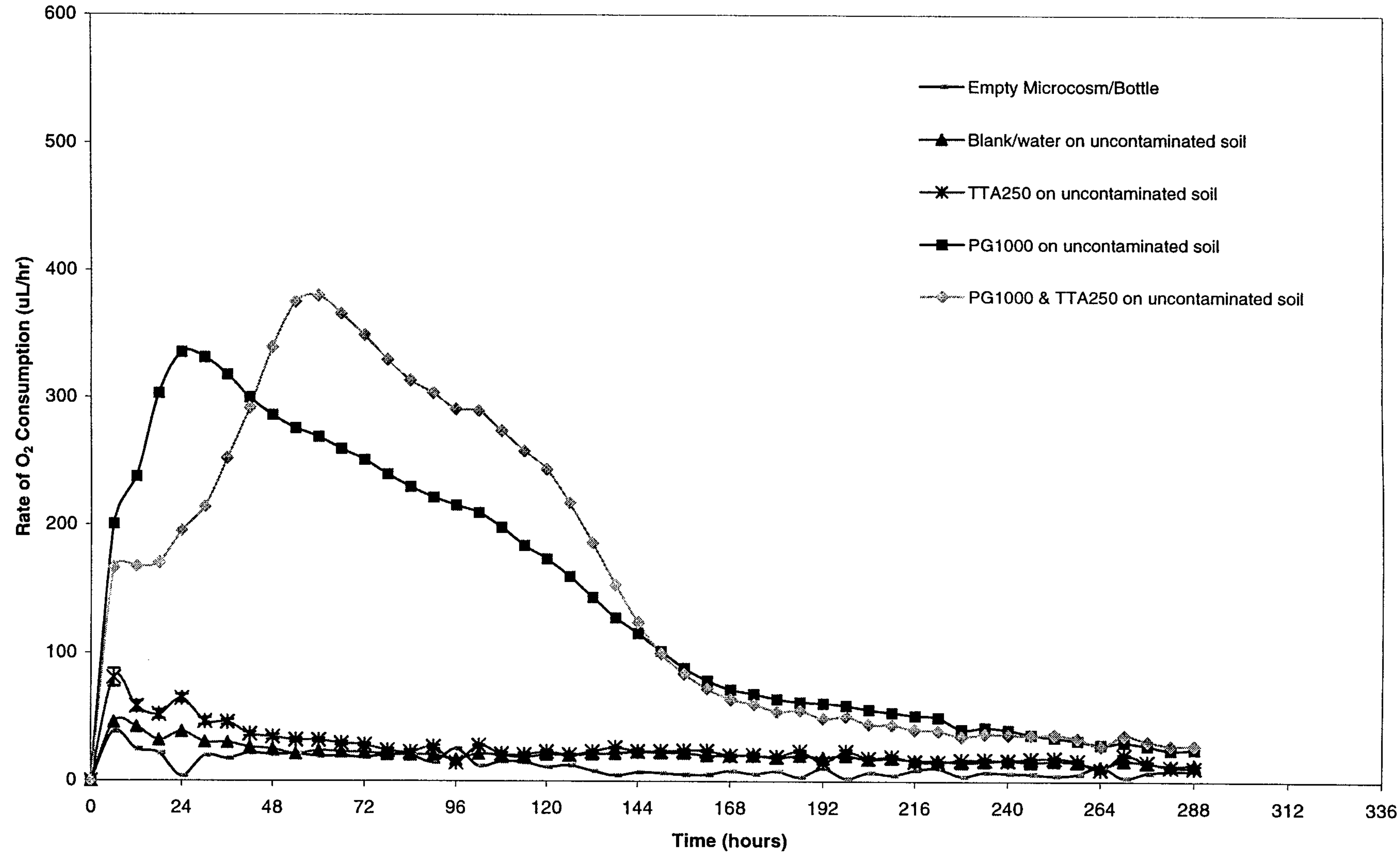


Figure E-8 Averaged Cumulative O_2 Consumption (uL), Experimental Run-2 (Re-accomplished)

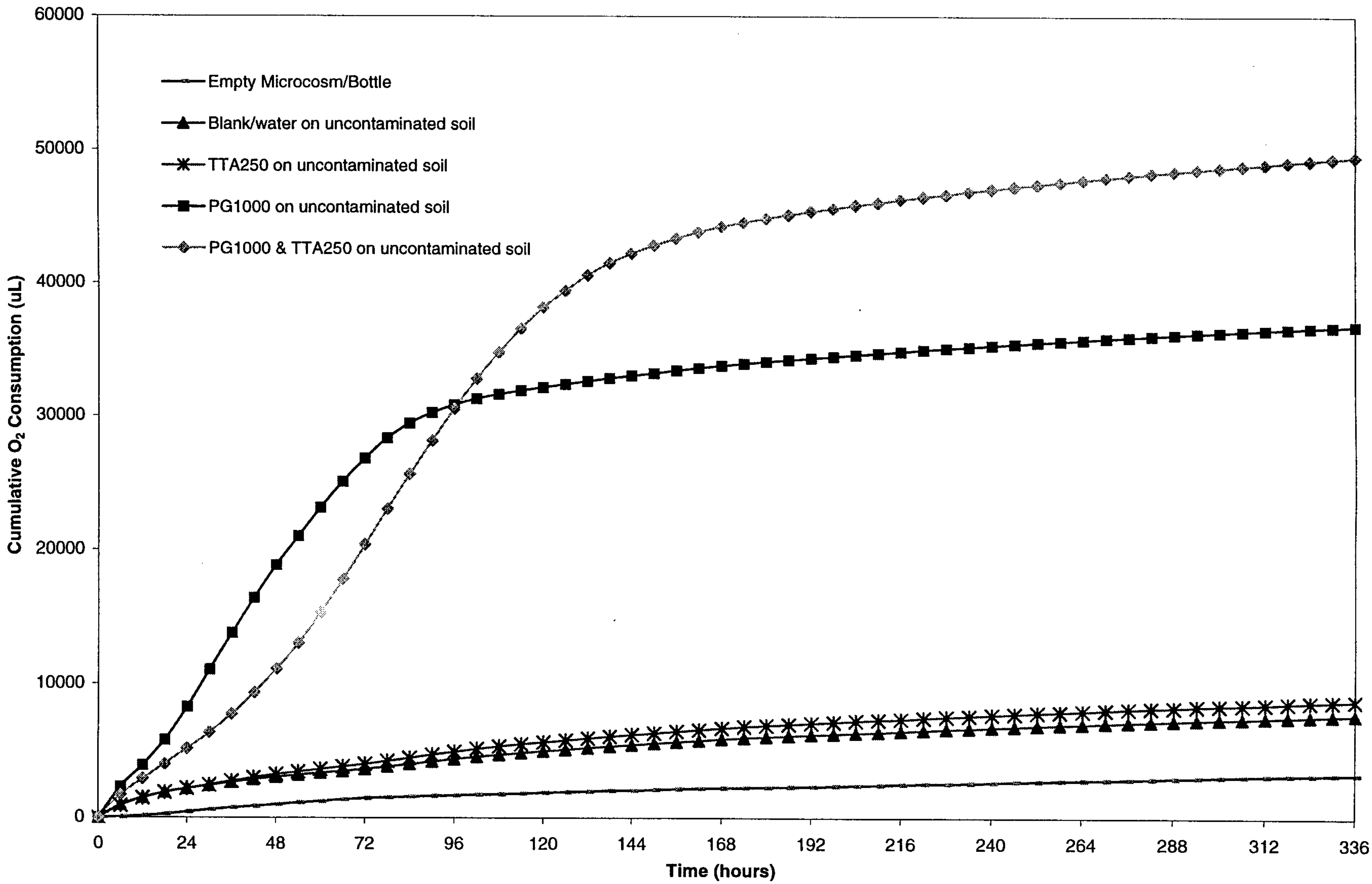


Figure E-9 Averaged Rate of O_2 Consumption (uL/hr), Experimental Run-2 (Re-accomplished)

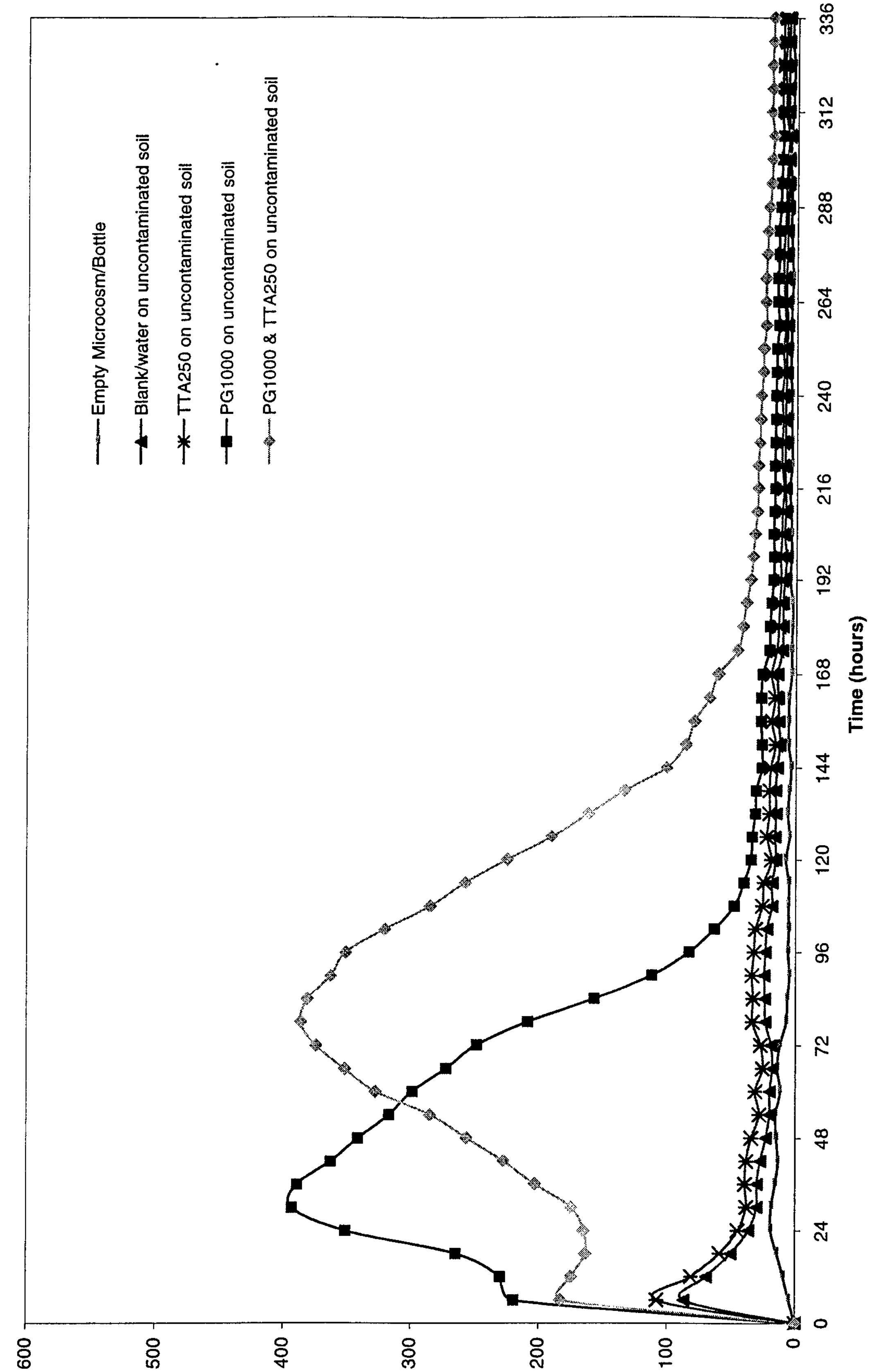


Figure E-10 Averaged Cumulative O_2 Consumption (uL), Experimental Run-3

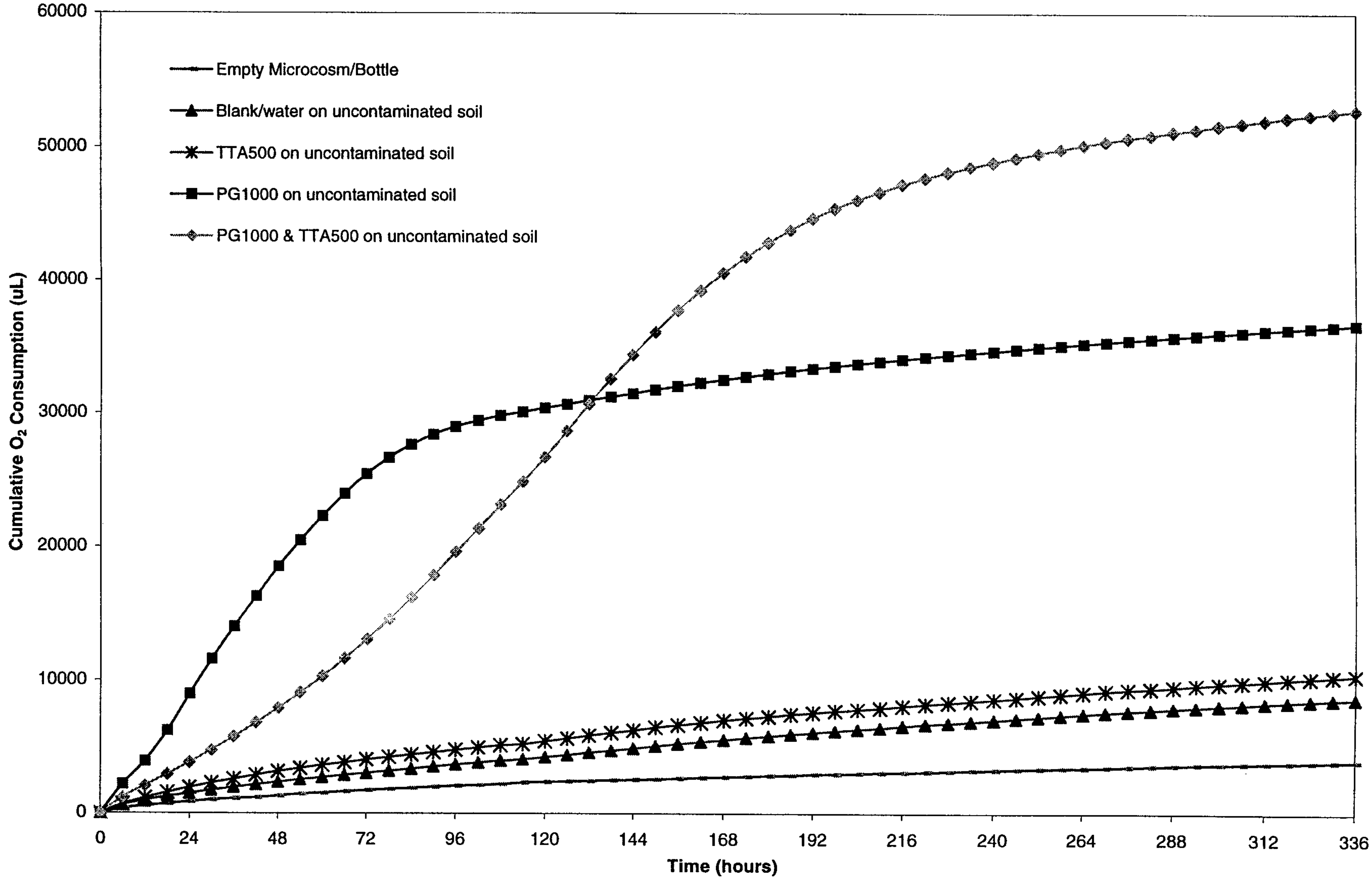


Figure E-11 Averaged Rate of O_2 Consumption (uL/hr), Experimental Run-3

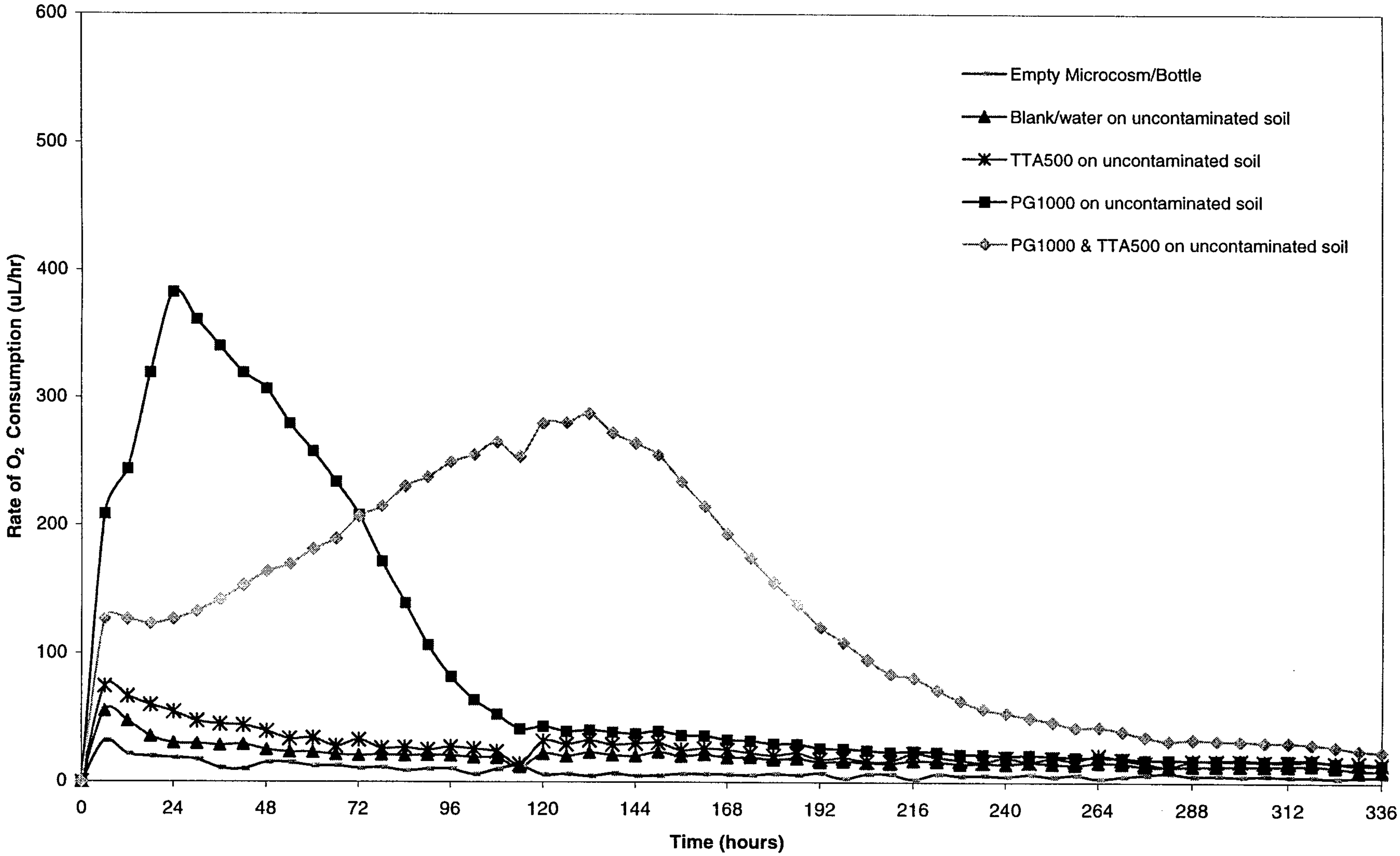


Figure E-12 Averaged Cumulative O_2 Consumption (uL), Experimental Run-4

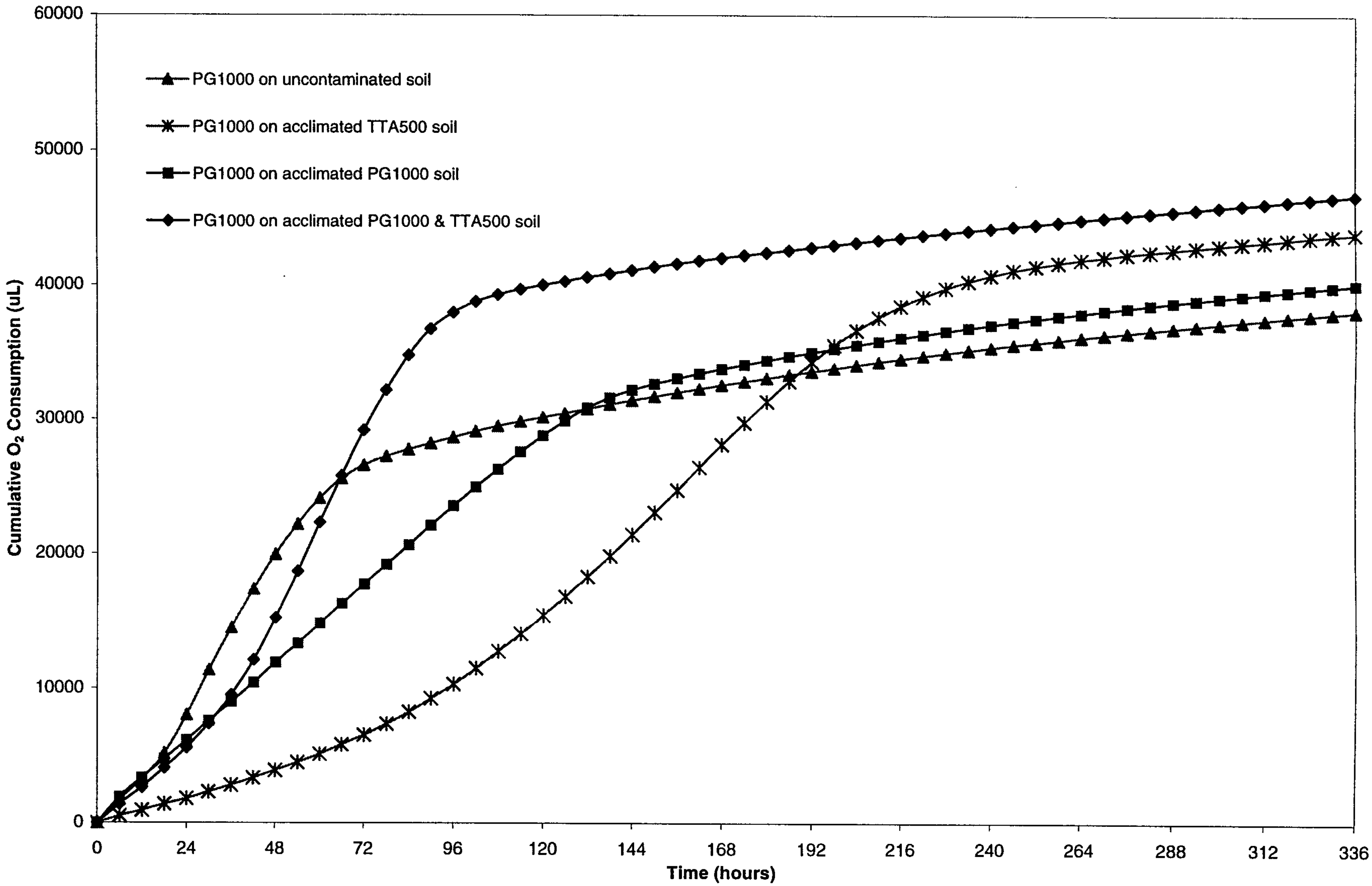


Figure E-13 Averaged Rate of O_2 Consumption (uL/hr), Experimental Run-4

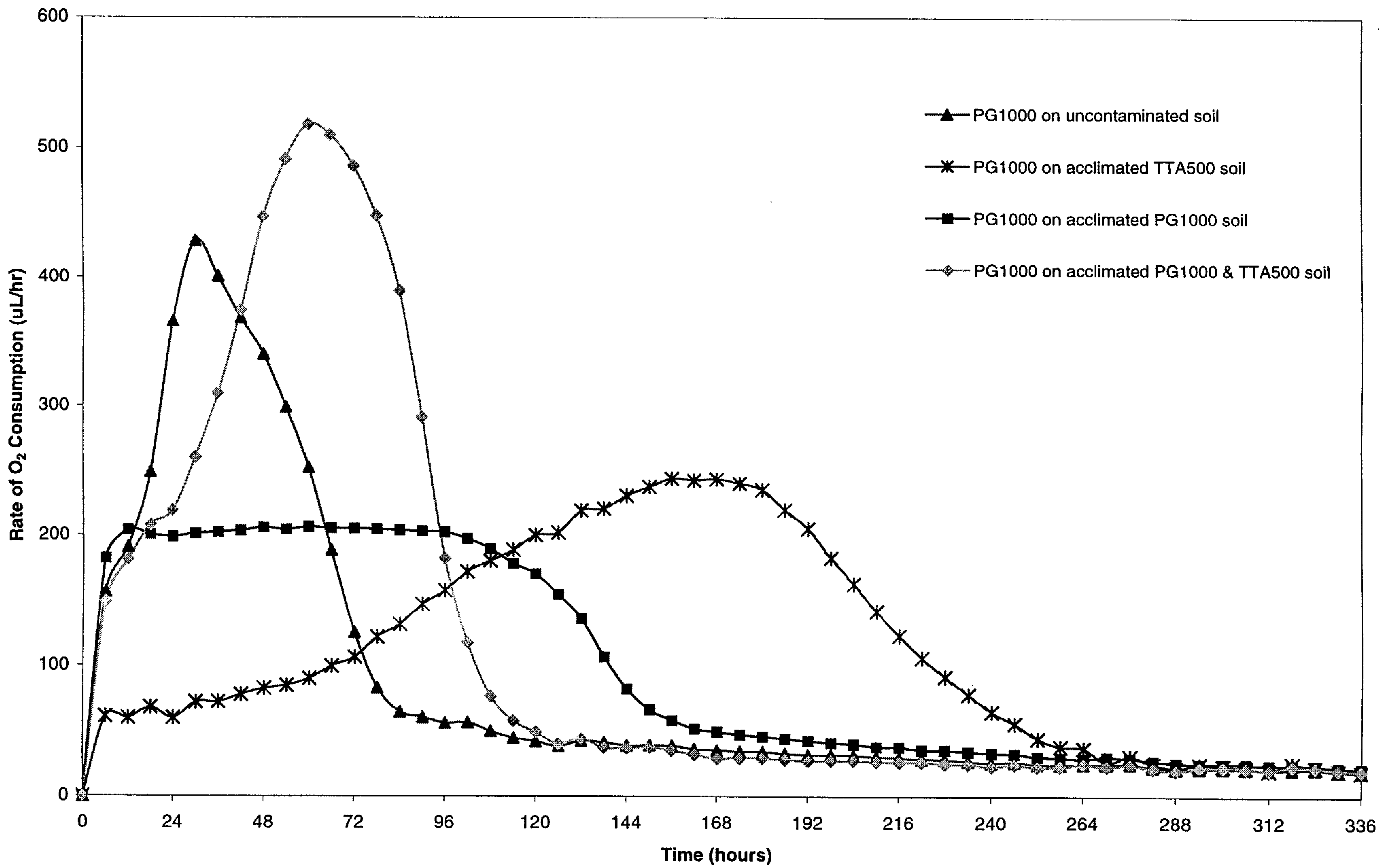


Figure E-14 Averaged Cumulative O_2 Consumption (uL), Experimental Run-5

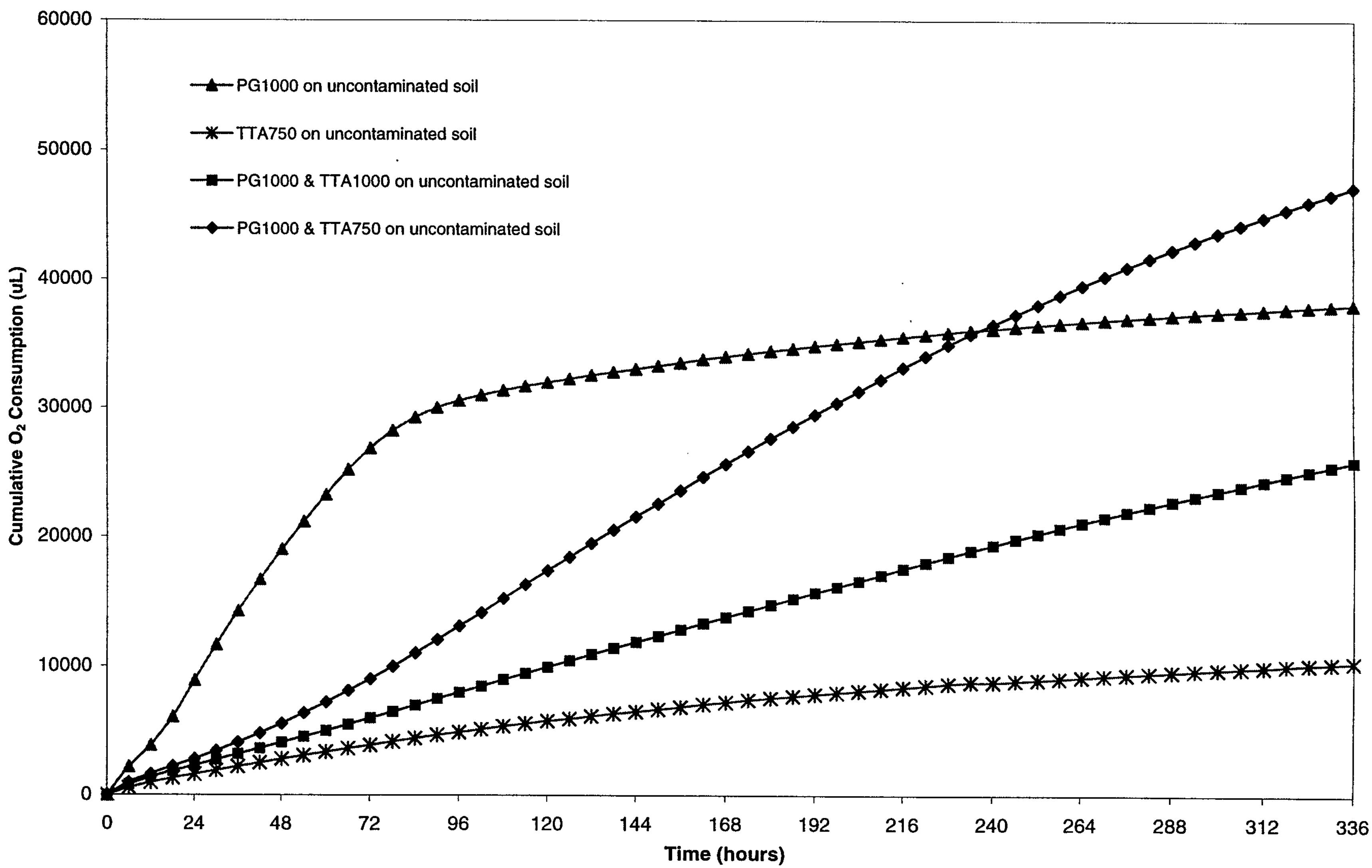


Figure E-15 Averaged Rate of O_2 Consumption (uL/hr), Experimental Run-5

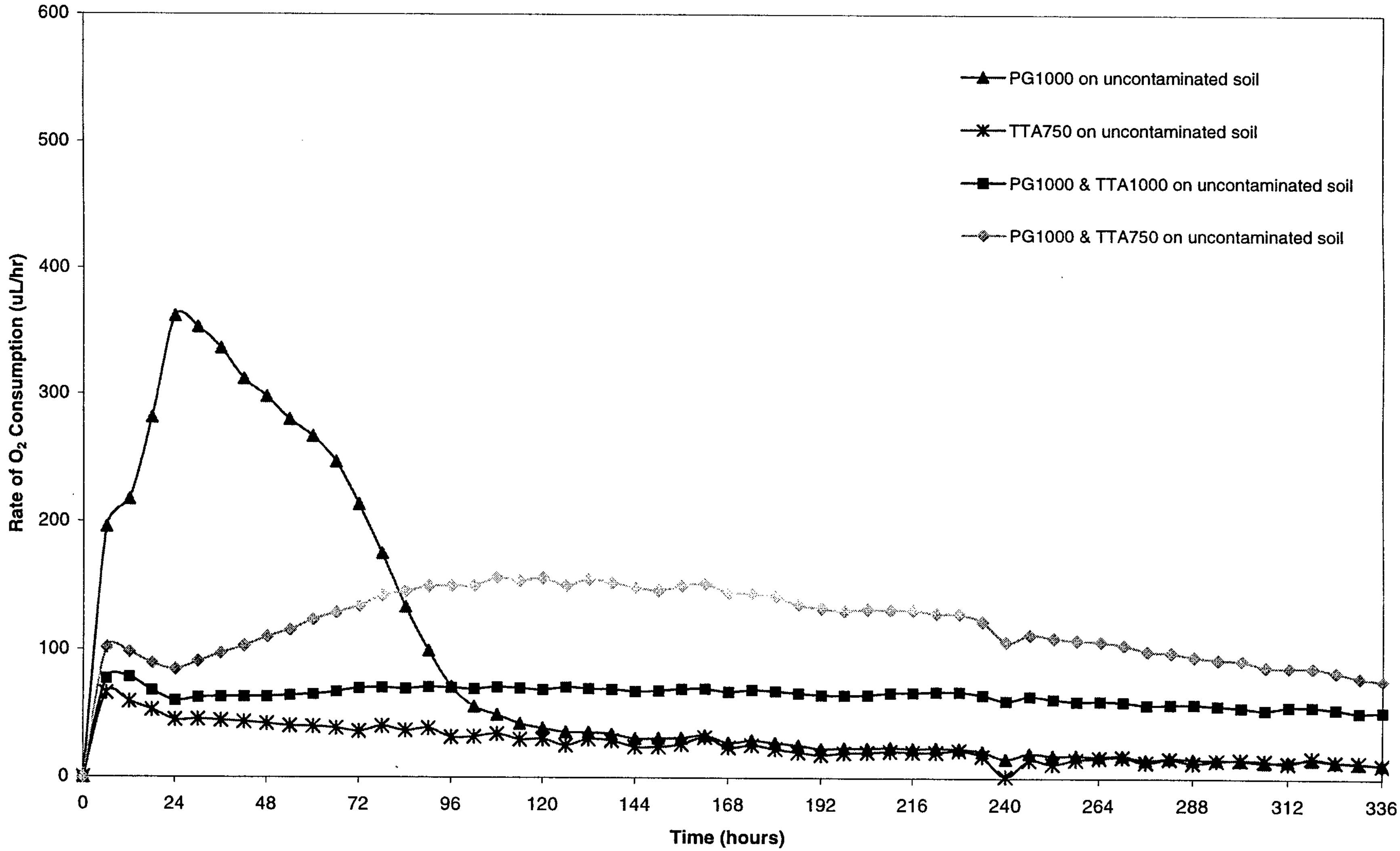


Figure E-16 Averaged Cumulative O_2 Consumption (uL), Experimental Run-6

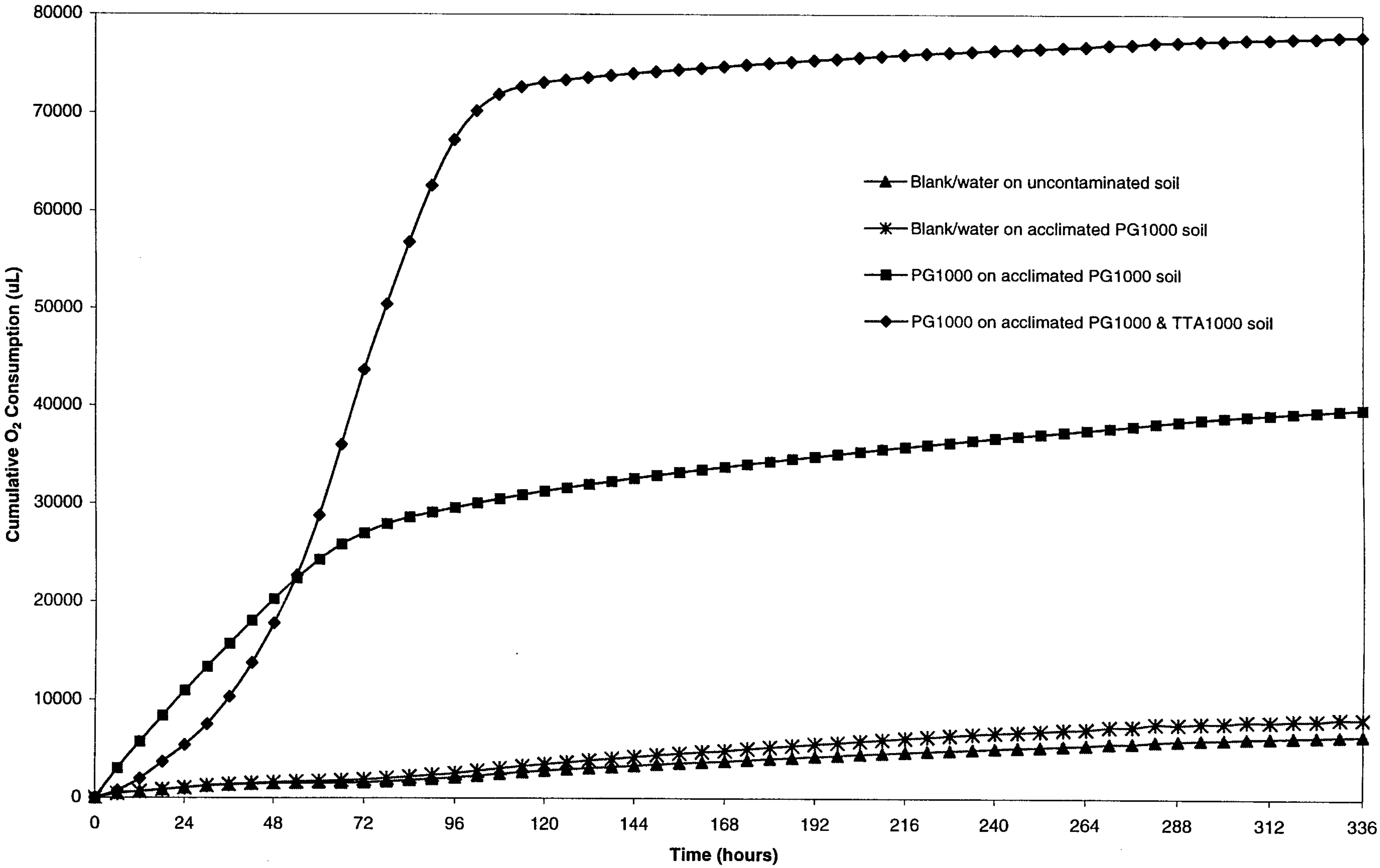
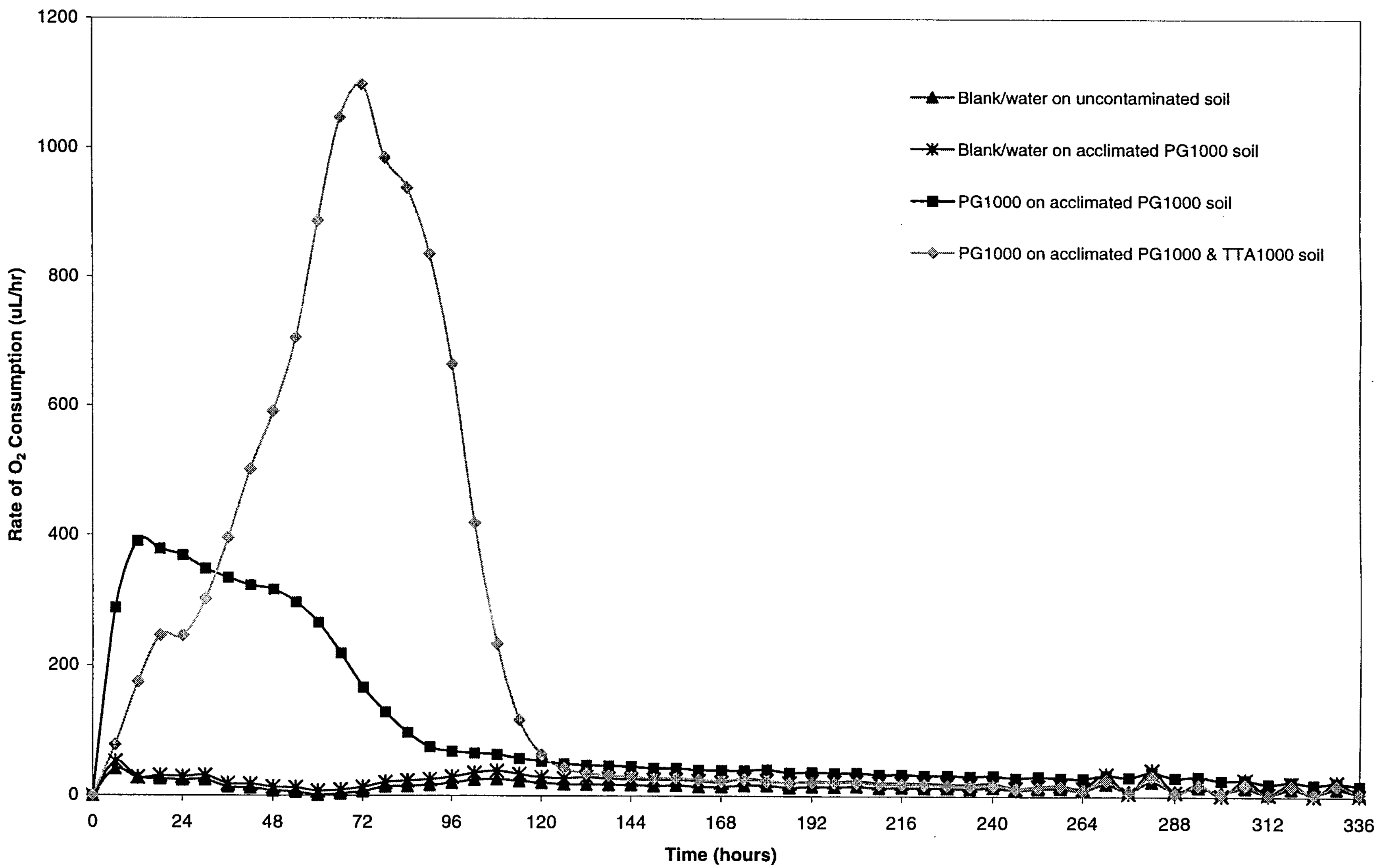


Figure E-17 Averaged Rate of O_2 Consumption (uL/hr), Experimental Run-6



Appendix F: Statistical Procedures for Determining Biodegradation Effects from the Addition of Individual ADF Chemicals (Propylene Glycol or Tolyltriazole) on Uncontaminated Soil

The data listed in the following five tables and figures explains the possible interaction (decreased/no influence/increased) of biodegradation from individual chemical components (PG or TTA) upon a soil environment. This determination was made using the O_2 consumption totals of the contaminated soil with (PG or TTA) against the uncontaminated soil. A two-sample t-test was performed using a significance level of $\alpha = 0.05$. A 95% CI was developed from the t-test results to depict the O_2 consumption effects. Both populations were assumed normal and the two population variances were assumed equal.

> H_o: There was no effect on the O_2 consumption due to the contaminant addition
> H_a: There was an effect (decreased or increased) on the O_2 consumption due to the contaminant addition

The pooled estimator, which is an estimate of the common population variance was determined by using the following equation (Devore, 358):

$$S_p^2 = \frac{(n_1-1)*S_1^2 + (n_2-1)*S_2^2}{(n_1+n_2) - 2}$$

Where n_1 and n_2 are the sample sizes of the respective treatments, and S_1 and S_2 are the standard deviations of the respective treatments.

The standard error was determined by the following equations (Devore, 358):

$$\text{Std-Error} = S_p \, (1/n_1+1/n_2)^{1/2}$$

The calculated t-statistic (t) was then determined by dividing the difference of the means by the standard error.

$$t = \frac{(X_{chemical} - X_{soil})}{(\text{Std-Error})}$$

The t-critical (t_{crit}) was determined for a two-tailed t-test since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis, thus $\alpha/2$ was used.

$$t_{crit} = t_{\alpha/2,\, n_1+n_2-2} = 2.447 \text{ (Devore, 707)}$$

Given: $\alpha = 0.05$ (95% confidence interval)
$n_1 = 3$ (number of blank microcosms)
$n_2 = 5$ (number of chemical microcosms)

The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical.

The t-critical (t_{crit}) was determined for a two-tailed test since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical. An example of the test statistic is shown below:

$t \leq -t_{crit}$	$t \leq -2.447$	Inhibition
$t \geq t_{crit}$	$t \geq 2.447$	Biodegradation

The upper and lower 95% CI were determined by using the following equation (Devore, 361). This data was shown with the difference of the means (for the sample at its particular position on the time line) in Figures F-1 through F-5.

Equation Format: $(X_{chemical} - X_{soil}) \pm (t_{\alpha/2,\, n_1+n_2-2}) * (S_p) * (1/n_1 + 1/n_2)^{1/2}$

X_{soil} = Uncontaminated soil is the control
$X_{chemical}$ = PG only -or- TTA only concentration amount

All observation points (every 6 hours) were statically tested for the entire respirometry period of 2 weeks.

List of Tables

List of Figures

Table F-1 (Run-1) Data (O_2) for Determining Biodegradation from the Individual Treatment of 25 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	Mean Soil (uL)	Std Dev Soil	Mean TTA25 in Soil (uL)	Std Dev TTA25 in Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA25} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0.000	0	0	NA
6	495	56	603	104	8233	66	108	1.628	270	-54	No Effect
12	818	72	1100	270	50445	164	282	1.720	683	-119	No Effect
18	1075	79	1529	422	120783	254	454	1.788	1075	-167	No Effect
24	1309	82	1908	569	218415	341	599	1.754	1434	-236	No Effect
30	1548	90	2263	730	357732	437	715	1.637	1784	-354	No Effect
36	1769	101	2620	876	515345	524	851	1.624	2134	-432	No Effect
42	1926	112	2847	1002	673928	600	922	1.537	2389	-545	No Effect
48	2049	113	3047	1138	868196	680	999	1.468	2664	-666	No Effect
54	2240	114	3349	1281	1097665	765	1109	1.449	2981	-763	No Effect
60	2431	112	3645	1417	1342338	846	1214	1.434	3284	-857	No Effect
66	2619	113	3929	1556	1619299	929	1310	1.410	3584	-964	No Effect
72	2797	119	4212	1690	1909109	1009	1415	1.402	3884	-1054	No Effect
78	2986	126	4477	1819	2210960	1086	1492	1.374	4149	-1166	No Effect
84	3186	132	4774	1934	2499896	1155	1587	1.375	4413	-1238	No Effect
90	3362	138	5033	2040	2782002	1218	1671	1.372	4651	-1310	No Effect
96	3518	143	5259	2136	3049086	1275	1740	1.365	4861	-1380	No Effect
102	3709	147	5547	2226	3310132	1329	1838	1.383	5089	-1414	No Effect
108	3880	151	5779	2313	3573859	1381	1899	1.375	5277	-1480	No Effect
114	4058	157	6015	2396	3836501	1430	1957	1.368	5457	-1543	No Effect
120	4238	162	6239	2476	4094449	1478	2002	1.354	5618	-1614	No Effect
126	4428	168	6477	2545	4327367	1519	2049	1.349	5767	-1668	No Effect
132	4622	172	6719	2611	4554081	1558	2096	1.345	5910	-1717	No Effect
138	4808	176	6940	2673	4774770	1596	2132	1.336	6037	-1773	No Effect
144	4996	182	7165	2730	4981328	1630	2169	1.331	6158	-1819	No Effect
150	5181	183	7389	2783	5175384	1661	2209	1.329	6274	-1857	No Effect
156	5366	188	7612	2837	5377402	1694	2246	1.326	6390	-1898	No Effect
162	5534	189	7808	2882	5549173	1720	2274	1.322	6484	-1935	No Effect
168	5705	196	8013	2925	5718301	1746	2308	1.321	6581	-1966	No Effect
174	5862	203	8204	2966	5880093	1771	2342	1.322	6675	-1991	No Effect
180	6015	206	8379	3009	6051805	1797	2364	1.316	6760	-2032	No Effect
186	6151	215	8531	3048	6207598	1820	2380	1.308	6833	-2072	No Effect
192	6277	219	8700	3083	6352236	1841	2423	1.316	6927	-2081	No Effect
198	6452	233	8928	3119	6501484	1862	2476	1.330	7033	-2081	No Effect
204	6595	237	9103	3153	6647500	1883	2507	1.332	7115	-2100	No Effect
210	6743	247	9271	3189	6801504	1905	2528	1.327	7189	-2132	No Effect
216	6888	259	9461	3222	6945322	1925	2573	1.337	7283	-2136	No Effect
222	7016	264	9595	3256	7091455	1945	2579	1.326	7338	-2180	No Effect
228	7149	267	9755	3288	7231037	1964	2606	1.327	7411	-2200	No Effect
234	7278	268	9891	3314	7346271	1979	2613	1.320	7457	-2230	No Effect
240	7404	272	10034	3341	7467582	1996	2629	1.317	7513	-2254	No Effect
246	7517	276	10157	3369	7594325	2013	2640	1.312	7565	-2285	No Effect
252	7627	282	10271	3398	7723754	2030	2645	1.303	7611	-2322	No Effect
258	7739	286	10374	3424	7842578	2045	2635	1.288	7639	-2370	No Effect
264	7849	288	10508	3447	7947922	2059	2659	1.292	7698	-2379	No Effect

Table F-1 (Run-1) Data (O_2) for Determining Biodegradation from the Individual Treatment of 25 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	Mean Soil (uL)	Std Dev Soil	Mean TTA25 in Soil (uL)	Std Dev TTA25 in Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA25} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
270	7962	297	10619	3475	8077715	2076	2657	1.280	7736	-2422	No Effect
276	8066	308	10721	3500	8196647	2091	2655	1.270	7771	-2461	No Effect
282	8166	314	10819	3527	8324805	2107	2653	1.259	7809	-2503	No Effect
288	8264	320	10922	3551	8441319	2122	2658	1.253	7850	-2534	No Effect
294	8359	331	11016	3575	8555635	2136	2657	1.244	7885	-2570	No Effect
300	8462	337	11117	3598	8670291	2150	2655	1.235	7917	-2607	No Effect
306	8555	343	11207	3622	8786387	2165	2653	1.225	7950	-2644	No Effect
312	8643	355	11302	3645	8897176	2178	2659	1.221	7989	-2672	No Effect
318	8727	360	11386	3669	9016700	2193	2659	1.213	8025	-2707	No Effect
324	8820	370	11501	3691	9127716	2206	2682	1.215	8081	-2717	No Effect
330	8910	373	11598	3712	9230573	2219	2688	1.212	8118	-2741	No Effect
336	8992	373	11684	3732	9329889	2231	2692	1.207	8151	-2766	No Effect

Figure F-1 Difference Between the Means (O_2) and 95% CI for 25 mg/kg Tolyltriazole on Uncontaminated Soil

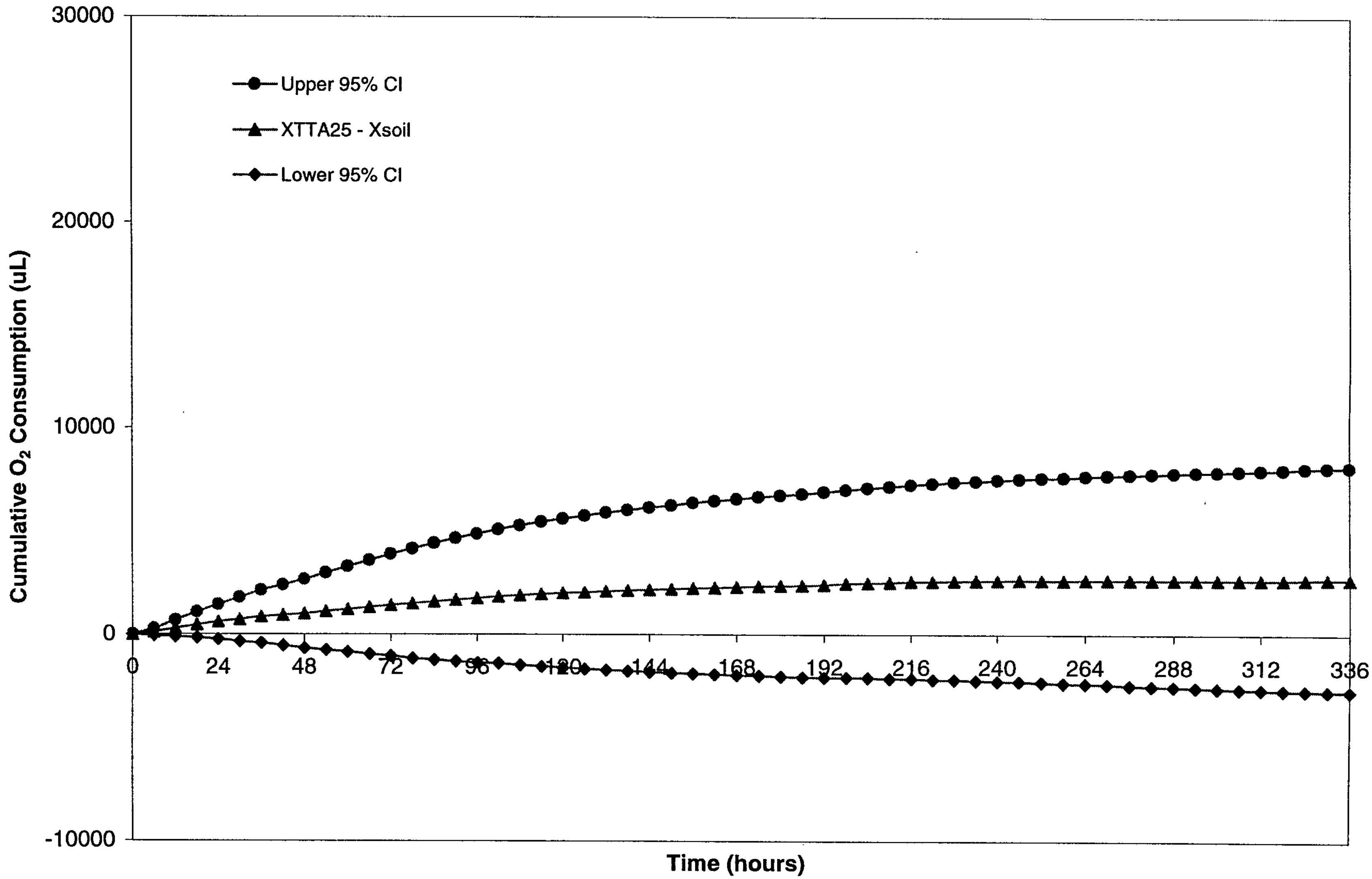


Table F-2 (Run-2) Data (O_2) for Determining Biodegradation from the Individual Treatment of 250 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	Mean Soil (uL)	Std Dev Soil	Mean TTA250 in Soil (uL)	Std Dev TTA500 in Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA250} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0.000	0	0	NA
6	960	90	907	80	7002	61	-53	-0.865	97	-202	no effect
12	1502	146	1470	135	19217	101	-33	-0.323	215	-280	no effect
18	1894	201	1877	185	36265	139	-17	-0.123	323	-357	no effect
24	2180	243	2188	225	53433	169	8	0.048	421	-405	no effect
30	2414	268	2453	258	68171	191	39	0.204	506	-428	no effect
36	2649	285	2726	291	83561	211	77	0.366	594	-439	no effect
42	2857	292	2993	322	97718	228	136	0.595	694	-423	no effect
48	3038	296	3232	352	111489	244	194	0.795	790	-403	no effect
54	3196	296	3424	377	124088	257	229	0.889	858	-401	no effect
60	3347	297	3642	403	137446	271	295	1.089	957	-368	no effect
66	3483	300	3818	425	150249	283	336	1.185	1028	-357	no effect
72	3632	298	4002	438	157468	290	371	1.279	1080	-338	no effect
78	3817	294	4237	461	170386	301	420	1.393	1157	-318	no effect
84	4006	294	4468	482	183631	313	462	1.475	1227	-304	no effect
90	4198	291	4697	492	189830	318	500	1.571	1278	-279	no effect
96	4382	285	4923	513	202206	328	541	1.646	1344	-263	no effect
102	4557	279	5140	529	212849	337	583	1.730	1407	-242	no effect
108	4700	273	5321	547	224300	346	621	1.796	1467	-225	no effect
114	4843	269	5493	563	235025	354	650	1.836	1516	-216	no effect
120	4963	258	5625	577	244428	361	662	1.834	1546	-221	no effect
126	5090	250	5780	589	252258	367	691	1.883	1588	-207	no effect
132	5210	244	5922	598	258305	371	712	1.919	1621	-196	no effect
138	5334	236	6066	610	266503	377	732	1.941	1654	-191	no effect
144	5448	230	6200	617	271124	380	752	1.977	1682	-179	no effect
150	5548	220	6311	630	280341	387	763	1.974	1710	-183	no effect
156	5652	211	6439	635	283773	389	787	2.022	1739	-165	no effect
162	5762	202	6551	642	288370	392	789	2.012	1749	-171	no effect
168	5875	192	6682	647	291284	394	808	2.049	1772	-157	no effect
174	5960	185	6789	655	297631	398	829	2.079	1803	-146	no effect
180	6038	176	6882	659	299707	400	844	2.112	1823	-134	no effect
186	6117	163	6973	665	303815	403	856	2.126	1841	-129	no effect
192	6188	155	7055	666	303737	402	867	2.155	1852	-118	no effect
198	6251	148	7141	675	311043	407	890	2.185	1886	-107	no effect
204	6324	140	7221	681	316030	411	897	2.186	1902	-107	no effect
210	6389	131	7300	691	324156	416	911	2.190	1928	-107	no effect
216	6459	124	7370	696	328304	418	911	2.178	1935	-113	no effect
222	6532	116	7450	703	333838	422	917	2.174	1950	-115	no effect
228	6601	110	7531	711	340967	426	931	2.182	1974	-113	no effect
234	6669	105	7608	719	348592	431	939	2.177	1994	-117	no effect
240	6736	97	7688	728	356088	436	952	2.184	2018	-115	no effect
246	6800	90	7758	735	362956	440	958	2.177	2035	-119	no effect
252	6864	84	7832	743	370877	445	968	2.177	2056	-120	no effect
258	6921	81	7895	751	378442	449	975	2.170	2074	-125	no effect
264	6987	78	7964	758	385321	453	977	2.156	2086	-132	no effect

Table F-2 (Run-2) Data (O_2) for Determining Biodegradation from the Individual Treatment of 250 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	Mean Soil (uL)	Std Dev Soil	Mean TTA250 in Soil (uL)	Std Dev TTA500 in Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA250} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
270	7053	74	8036	766	392818	458	983	2.147	2103	-137	no effect
276	7114	73	8108	770	397438	460	994	2.160	2121	-132	no effect
282	7176	65	8175	777	404158	464	999	2.152	2135	-137	no effect
288	7232	61	8236	786	413350	470	1004	2.139	2153	-145	no effect
294	7284	56	8296	795	422532	475	1012	2.132	2174	-149	no effect
300	7336	53	8350	802	429812	479	1014	2.119	2186	-157	no effect
306	7382	49	8398	811	439111	484	1016	2.099	2200	-169	no effect
312	7439	44	8462	817	445275	487	1024	2.101	2216	-169	no effect
318	7494	43	8525	823	451792	491	1031	2.099	2232	-171	no effect
324	7549	47	8597	829	459385	495	1048	2.118	2260	-163	no effect
330	7604	49	8658	837	467993	500	1054	2.109	2276	-169	no effect
336	7654	53	8719	843	474905	503	1065	2.116	2296	-167	no effect

FIGURE F-2 Difference Between the Means (O_2) and 95% CI for 250 mg/kg Tolyltriazole on Uncontaminated Soil

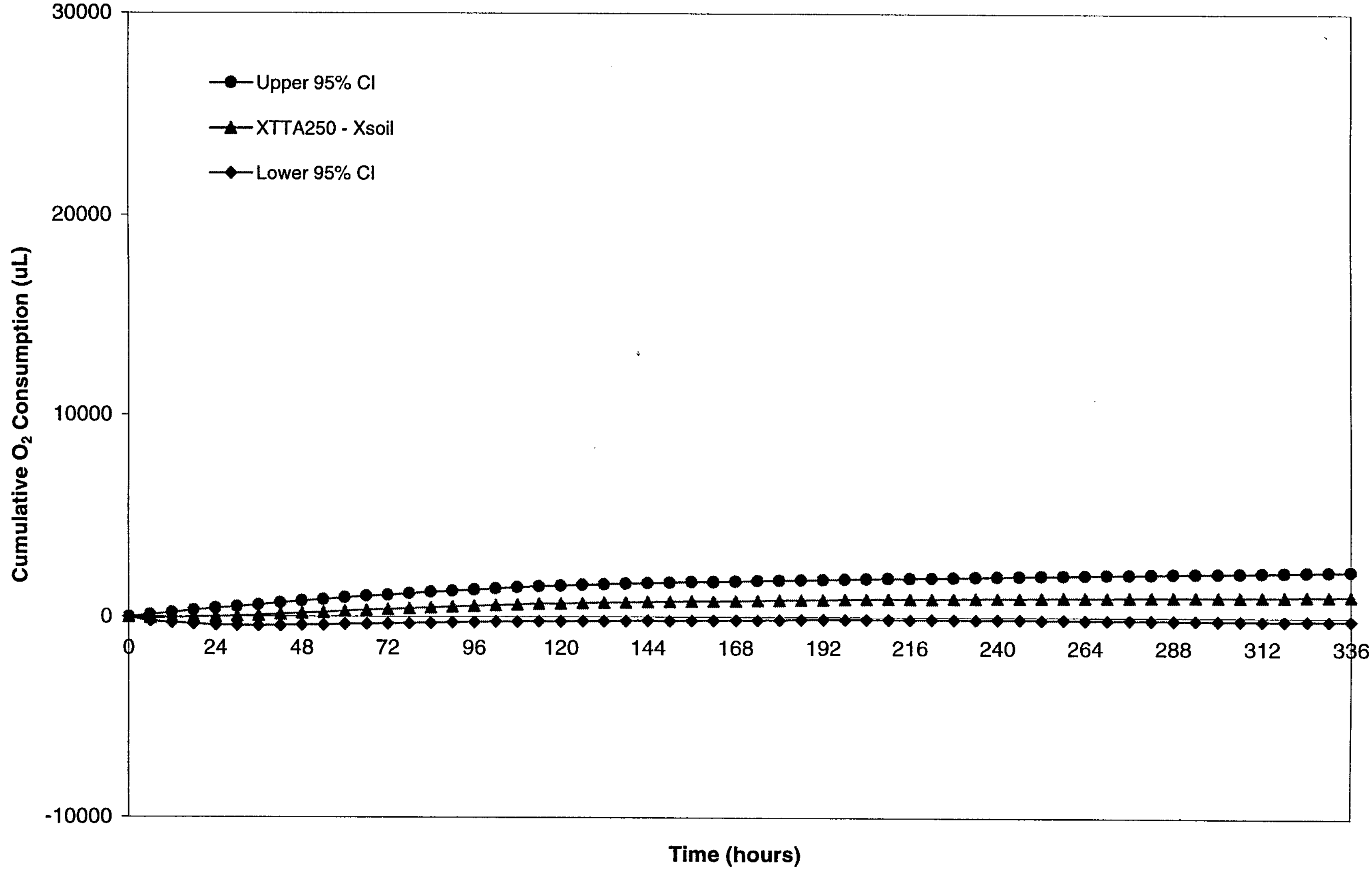


Table F-3 (Run-3) Data (O_2) for Determining Biodegradation from the Individual Treatment of 500 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	Mean Soil (uL)	Std Dev Soil	Mean TTA500 in Soil (uL)	Std Dev TTA500 in Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA500} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0.000	0	0	NA
6	610	25	683	37	1140	25	73	2.972	134	13	Biodegradation
12	981	29	1147	84	5027	52	166	3.198	292	39	Biodegradation
18	1258	34	1563	123	10398	74	305	4.096	487	123	Biodegradation
24	1493	36	1940	158	17075	95	447	4.684	680	213	Biodegradation
30	1725	43	2269	189	24411	114	544	4.768	823	265	Biodegradation
36	1949	48	2582	220	32978	133	634	4.777	958	309	Biodegradation
42	2169	35	2887	247	41113	148	718	4.849	1080	356	Biodegradation
48	2361	34	3161	271	49156	162	799	4.936	1195	403	Biodegradation
54	2544	40	3394	295	58353	176	850	4.817	1281	418	Biodegradation
60	2718	40	3631	316	67118	189	913	4.825	1376	450	Biodegradation
66	2878	48	3826	337	76580	202	948	4.689	1442	453	Biodegradation
72	3032	60	4053	359	86896	215	1020	4.740	1547	494	Biodegradation
78	3197	70	4232	372	93759	224	1035	4.629	1582	488	Biodegradation
84	3357	79	4417	391	103896	235	1060	4.504	1636	484	Biodegradation
90	3519	92	4590	409	114380	247	1071	4.334	1675	466	Biodegradation
96	3673	113	4779	424	124358	258	1106	4.295	1736	476	Biodegradation
102	3823	123	4957	442	135492	269	1134	4.219	1792	476	Biodegradation
108	3968	134	5117	457	145105	278	1149	4.129	1829	468	Biodegradation
114	4060	140	5210	467	151692	284	1150	4.044	1846	454	Biodegradation
120	4231	153	5428	485	164415	296	1197	4.042	1922	472	Biodegradation
126	4387	165	5630	498	174507	305	1242	4.073	1989	496	Biodegradation
132	4566	177	5850	514	186501	315	1284	4.072	2056	512	Biodegradation
138	4730	188	6053	536	203115	329	1323	4.021	2129	518	Biodegradation
144	4888	204	6256	549	214849	339	1368	4.041	2196	539	Biodegradation
150	5068	219	6468	568	230704	351	1400	3.991	2258	542	Biodegradation
156	5226	229	6641	583	243969	361	1415	3.922	2298	532	Biodegradation
162	5388	248	6822	599	260039	372	1433	3.849	2345	522	Biodegradation
168	5532	262	6996	615	274892	383	1464	3.822	2400	527	Biodegradation
174	5677	280	7150	630	290515	394	1473	3.743	2436	510	Biodegradation
180	5813	290	7290	641	301518	401	1477	3.684	2459	496	Biodegradation
186	5958	301	7448	654	315609	410	1490	3.631	2494	486	Biodegradation
192	6081	308	7572	667	327998	418	1491	3.565	2514	468	Biodegradation
198	6208	317	7704	679	341228	427	1496	3.507	2540	452	Biodegradation
204	6323	328	7817	694	356817	436	1494	3.425	2562	427	Biodegradation
210	6436	339	7929	705	369596	444	1493	3.362	2579	406	Biodegradation
216	6567	348	8081	717	383127	452	1515	3.351	2621	409	Biodegradation
222	6693	355	8213	724	390995	457	1520	3.328	2637	402	Biodegradation
228	6805	362	8329	733	401343	463	1525	3.295	2657	392	Biodegradation
234	6917	371	8438	745	416020	471	1520	3.228	2673	368	Biodegradation
240	7022	383	8570	758	432228	480	1548	3.223	2723	373	Biodegradation
246	7143	390	8679	772	447815	489	1536	3.143	2732	340	Biodegradation
252	7256	397	8806	779	457464	494	1550	3.137	2758	341	Biodegradation
258	7362	405	8923	792	472756	502	1560	3.107	2789	332	Biodegradation
264	7482	410	9065	802	484486	508	1582	3.113	2826	339	Biodegradation

Table F-3 (Run-3) Data (O_2) for Determining Biodegradation from the Individual Treatment of 500 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	Mean Soil (uL)	Std Dev Soil	Mean TTA500 in Soil (uL)	Std Dev TTA500 in Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA500} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
270	7593	422	9189	812	498587	516	1596	3.096	2858	335	Biodegradation
276	7688	432	9294	823	513552	523	1606	3.068	2886	325	Biodegradation
282	7782	438	9379	833	526909	530	1597	3.013	2894	300	Biodegradation
288	7881	448	9497	845	542917	538	1615	3.002	2932	299	Biodegradation
294	7980	454	9612	855	556176	545	1632	2.997	2965	299	Biodegradation
300	8081	462	9725	862	566064	549	1643	2.991	2988	299	Biodegradation
306	8180	470	9837	869	577373	555	1657	2.986	3015	299	Biodegradation
312	8277	482	9949	882	596556	564	1672	2.964	3052	292	Biodegradation
318	8382	493	10065	889	608021	569	1683	2.956	3077	290	Biodegradation
324	8476	500	10172	899	622033	576	1696	2.944	3105	286	Biodegradation
330	8549	511	10279	906	633967	581	1730	2.976	3153	308	Biodegradation
336	8624	514	10380	912	642748	585	1756	2.999	3188	323	Biodegradation

Figure F-3 Difference Between the Means (O_2) and 95% CI for 500 mg/kg Tolyltriazole on Uncontaminated Soil

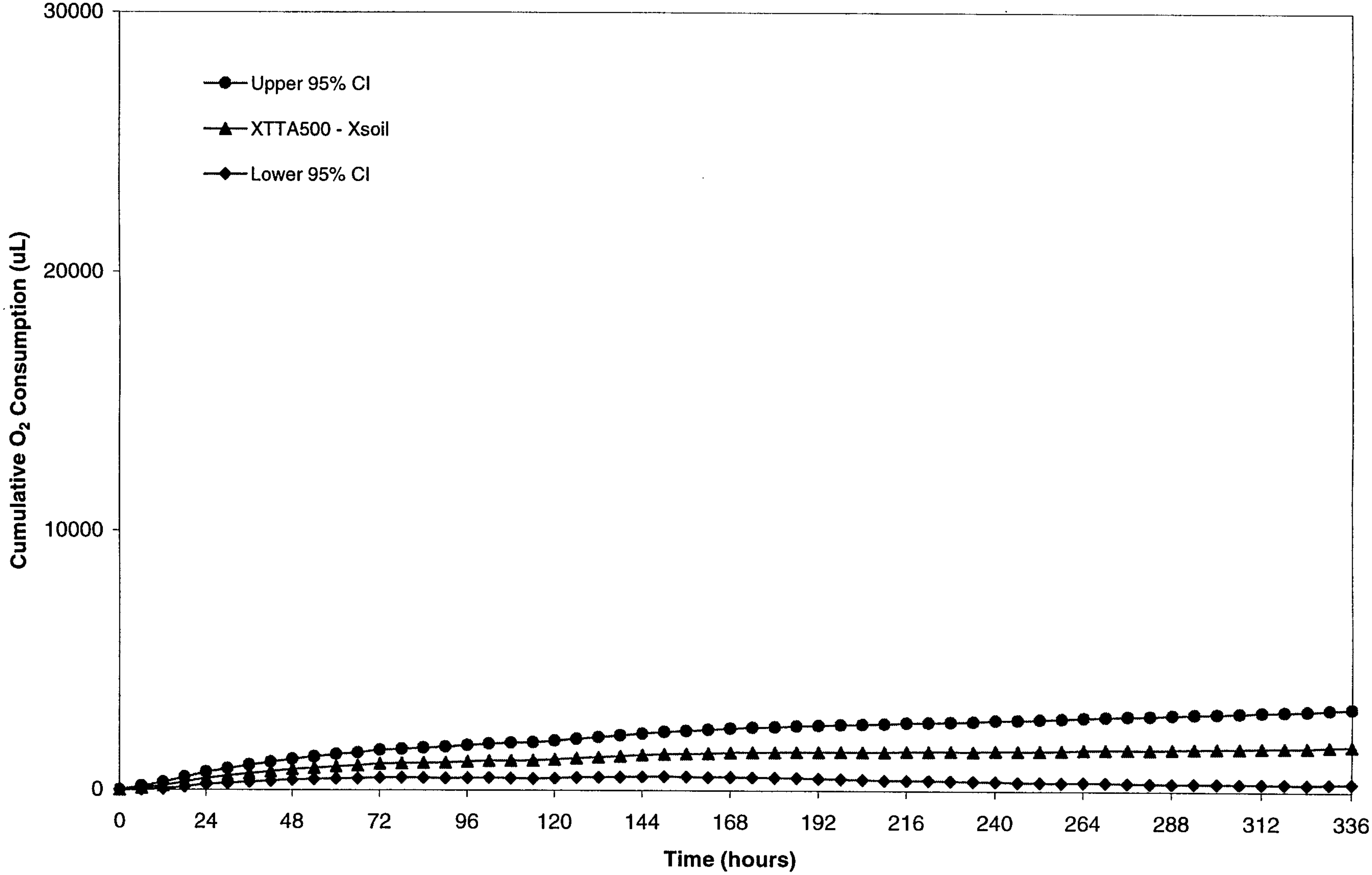


Table F-4 (Run-5) Data (O_2) for Determining Biodegradation from the Individual Treatment of 750 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	(RUN-3 Used) Mean Soil (uL)	(RUN-3 Used) Std Dev Soil	Mean TTA750 In Soil (uL)	Std Dev TTA750 In Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA750} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0.000	0	0	NA
6	610	25	553	14	342	14	-56	-4.174	-23	-89	Biodegradation
12	981	29	950	24	657	19	-31	-1.640	15	-76	Biodegradation
18	1258	34	1306	44	1646	30	48	1.621	121	-24	Biodegradation
24	1493	36	1608	60	2844	39	115	2.956	210	20	Biodegradation
30	1725	43	1913	77	4586	49	188	3.801	309	67	Biodegradation
36	1949	48	2212	93	6547	59	263	4.450	408	118	Biodegradation
42	2169	35	2505	106	7974	65	335	5.142	495	176	Biodegradation
48	2361	34	2796	110	8470	67	434	6.464	599	270	Biodegradation
54	2544	40	3075	118	9765	72	531	7.360	708	355	Biodegradation
60	2718	40	3351	124	10750	76	633	8.354	818	447	Biodegradation
66	2878	48	3613	134	12708	82	735	8.927	936	534	Biodegradation
72	3032	60	3864	140	14216	87	831	9.549	1045	618	Biodegradation
78	3197	70	4141	142	15089	90	944	10.529	1164	725	Biodegradation
84	3357	79	4395	150	17038	95	1038	10.890	1271	805	Biodegradation
90	3519	92	4658	156	19037	101	1139	11.304	1386	892	Biodegradation
96	3673	113	4877	158	20837	105	1204	11.419	1462	946	Biodegradation
102	3823	123	5098	160	22067	108	1276	11.759	1541	1010	Biodegradation
108	3968	134	5329	170	25284	116	1361	11.724	1646	1077	Biodegradation
114	4060	140	5531	178	27518	121	1471	12.144	1768	1175	Biodegradation
120	4231	153	5739	183	30251	127	1508	11.872	1819	1197	Biodegradation
126	4387	165	5910	191	33325	133	1523	11.422	1849	1197	Biodegradation
132	4566	177	6115	203	37879	142	1549	10.895	1896	1201	Biodegradation
138	4730	188	6311	217	43254	152	1581	10.412	1953	1210	Biodegradation
144	4888	204	6475	228	48604	161	1587	9.858	1981	1193	Biodegradation
150	5068	219	6639	241	54686	171	1570	9.193	1988	1152	Biodegradation
156	5226	229	6816	255	60835	180	1590	8.829	2031	1149	Biodegradation
162	5388	248	7030	272	69896	193	1642	8.504	2114	1170	Biodegradation
168	5532	262	7195	283	76078	201	1663	8.255	2156	1170	Biodegradation
174	5677	280	7373	295	84225	212	1696	8.003	2215	1178	Biodegradation
180	5813	290	7530	305	90101	219	1717	7.831	2253	1180	Biodegradation
186	5958	301	7668	312	95253	225	1710	7.587	2262	1159	Biodegradation
192	6081	308	7793	320	100104	231	1712	7.411	2278	1147	Biodegradation
198	6208	317	7929	329	105723	237	1721	7.247	2302	1140	Biodegradation
204	6323	328	8068	340	113182	246	1745	7.103	2346	1144	Biodegradation
210	6436	339	8212	350	119870	253	1776	7.022	2394	1157	Biodegradation
216	6567	348	8352	361	127265	261	1786	6.854	2423	1148	Biodegradation
222	6693	355	8493	372	134074	267	1800	6.731	2454	1146	Biodegradation
228	6805	362	8641	383	141616	275	1836	6.681	2509	1164	Biodegradation
234	6917	371	8761	393	149020	282	1844	6.539	2533	1154	Biodegradation
240	7022	383	8752	393	151867	285	1730	6.078	2426	1033	Biodegradation
246	7143	390	8849	399	156905	289	1706	5.897	2414	998	Biodegradation
252	7256	397	8927	403	161105	293	1671	5.700	2388	954	Biodegradation
258	7362	405	9029	419	171688	303	1667	5.509	2407	926	Biodegradation
264	7482	410	9140	426	176969	307	1658	5.397	2410	906	Biodegradation

Table F-4 (Run-5) Data (O_2) for Determining Biodegradation from the Individual Treatment of 750 mg/kg Tolyltriazole on Uncontaminated Soil

Time (hours)	(RUN-3 Used) Mean Soil (uL)	(RUN-3 Used) Std Dev Soil	Mean TTA750 In Soil (uL)	Std Dev TTA750 In Soil	Pooled Estimator S_p^2	Standard Error	$X_{TTA750} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
270	7593	422	9257	435	185559	315	1664	5.289	2434	894	Biodegradation
276	7688	432	9344	442	192173	320	1656	5.172	2439	873	Biodegradation
282	7782	438	9452	449	198389	325	1670	5.133	2466	874	Biodegradation
288	7881	448	9537	455	204497	330	1656	5.014	2464	848	Biodegradation
294	7980	454	9635	461	210630	335	1655	4.938	2475	835	Biodegradation
300	8081	462	9737	470	218816	342	1656	4.848	2492	820	Biodegradation
306	8180	470	9835	477	225663	347	1655	4.770	2504	806	Biodegradation
312	8277	482	9920	484	233501	353	1643	4.655	2506	779	Biodegradation
318	8382	493	10032	491	241905	359	1651	4.596	2530	772	Biodegradation
324	8476	500	10126	498	248862	364	1650	4.529	2542	759	Biodegradation
330	8549	511	10215	504	256490	370	1666	4.504	2571	761	Biodegradation
336	8624	514	10290	512	263235	375	1666	4.447	2583	749	Biodegradation

Figure F-4 Difference Between the Means (O_2) and 95% CI for 750 mg/kg Tolyltriazole on Uncontaminated Soil

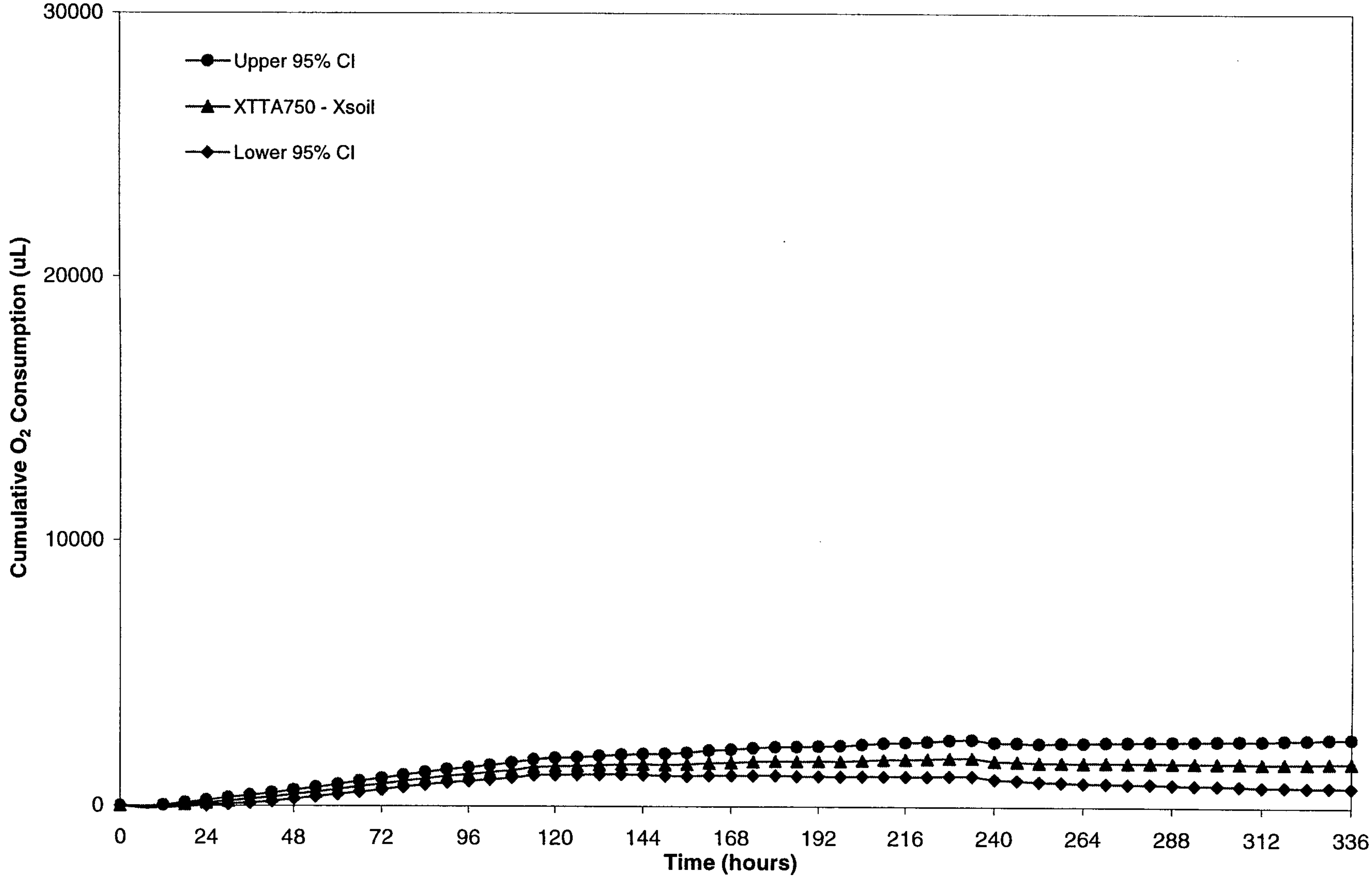


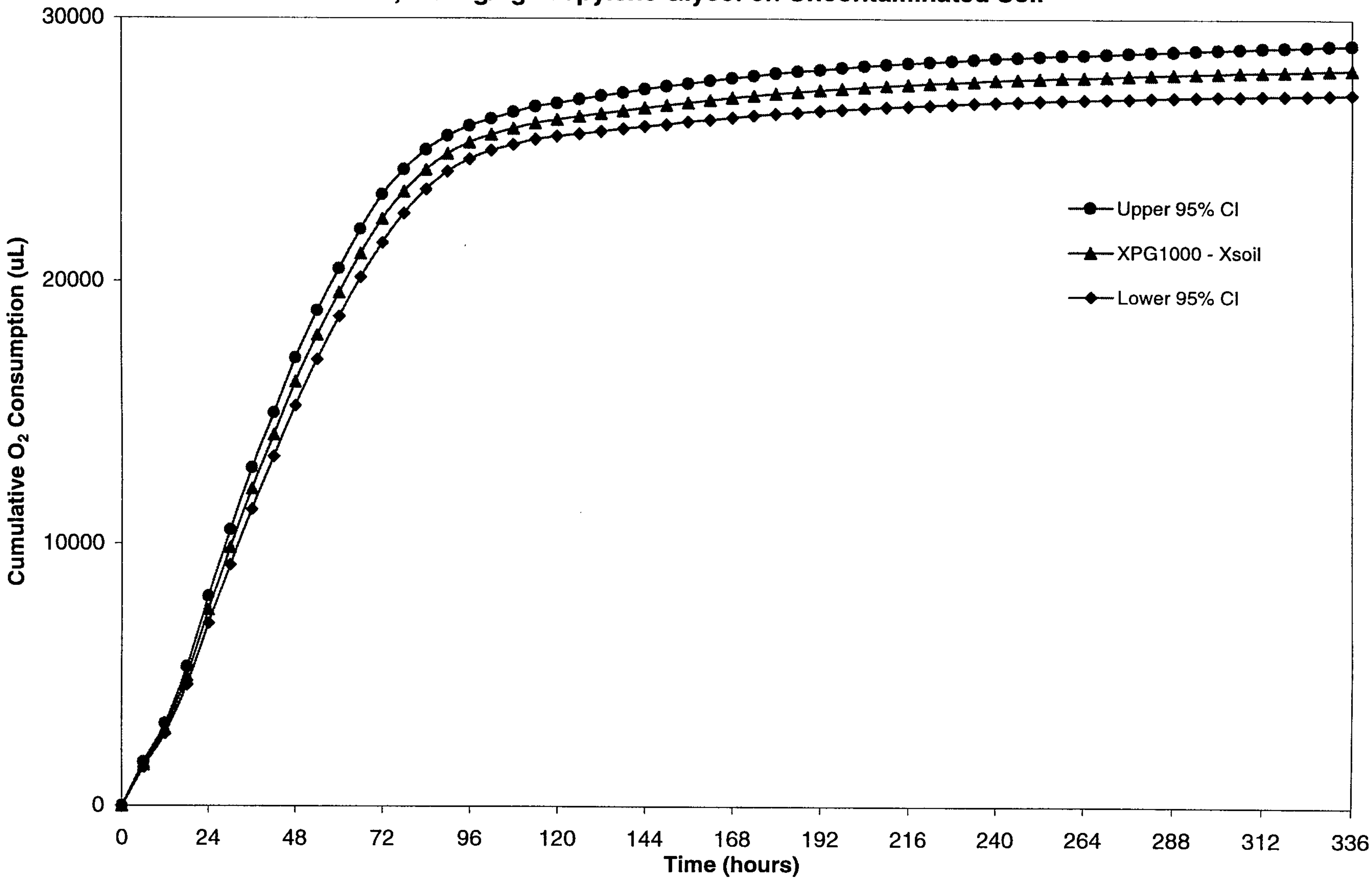
Table F-5 (Run-3) Data (O_2) for Determining Biodegradation from Individual Treatment of 1,000 mg/kg Propylene Glycol on Uncontaminated Soil

Time (hrs)	Mean Soil (uL)	Std Dev Soil	Mean PG1000 In Soil (uL)	Std Dev PG1000 In Soil	Pooled Estimator S_p^2	Standard Error	$X_{PG1000} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0.000	0	0	N/A
6	610	25	2189	61	2715	38	1580	41.508	1673	1486	Biodegradation
12	981	29	3944	124	10591	75	2963	39.421	3147	2779	Biodegradation
18	1258	34	6240	233	36640	140	4982	35.641	5324	4640	Biodegradation
24	1493	36	8994	354	83807	211	7501	35.479	8018	6984	Biodegradation
30	1725	43	11593	452	136897	270	9868	36.520	10529	9207	Biodegradation
36	1949	48	14043	539	194723	322	12094	37.530	12883	11306	Biodegradation
42	2169	35	16312	568	215803	339	14142	41.686	14972	13312	Biodegradation
48	2361	34	18520	622	258077	371	16159	43.554	17066	15251	Biodegradation
54	2544	40	20489	638	271553	381	17945	47.154	18876	17014	Biodegradation
60	2718	40	22295	624	260307	373	19577	52.541	20489	18665	Biodegradation
66	2878	48	23960	630	265562	376	21082	56.017	22002	20161	Biodegradation
72	3032	60	25442	630	265505	376	22410	59.553	23331	21489	Biodegradation
78	3197	70	26650	571	219015	342	23453	68.622	24289	22617	Biodegradation
84	3357	79	27646	513	177705	308	24289	78.897	25042	23536	Biodegradation
90	3519	92	28411	456	141552	275	24892	90.595	25564	24220	Biodegradation
96	3673	113	28994	421	122396	255	25321	99.106	25946	24696	Biodegradation
102	3823	123	29441	398	110786	243	25618	105.392	26213	25023	Biodegradation
108	3968	134	29819	407	116417	249	25851	103.747	26461	25242	Biodegradation
114	4060	140	30113	415	121176	254	26053	102.482	26675	25431	Biodegradation
120	4231	153	30413	414	121862	255	26182	102.700	26806	25558	Biodegradation
126	4387	165	30696	431	132795	266	26309	98.858	26960	25658	Biodegradation
132	4566	177	30983	444	142109	275	26417	95.957	27091	25744	Biodegradation
138	4730	188	31253	445	144025	277	26523	95.699	27201	25845	Biodegradation
144	4888	204	31524	460	155137	288	26636	92.599	27340	25932	Biodegradation
150	5068	219	31806	470	162946	295	26738	90.699	27459	26016	Biodegradation
156	5226	229	32059	466	162143	294	26833	91.249	27553	26114	Biodegradation
162	5388	248	32318	477	172250	303	26930	88.849	27671	26188	Biodegradation
168	5532	262	32553	478	175225	306	27021	88.389	27769	26273	Biodegradation
174	5677	280	32777	475	176759	307	27100	88.265	27852	26349	Biodegradation
180	5813	290	32992	482	182957	312	27179	87.008	27943	26415	Biodegradation
186	5958	301	33204	489	189888	318	27245	85.614	28024	26467	Biodegradation
192	6081	308	33389	487	189993	318	27308	85.788	28087	26529	Biodegradation
198	6208	317	33576	493	195714	323	27368	84.710	28159	26578	Biodegradation
204	6323	328	33754	497	200726	327	27431	83.838	28232	26631	Biodegradation
210	6436	339	33919	495	201449	328	27482	83.844	28285	26680	Biodegradation
216	6567	348	34093	501	207508	333	27526	82.743	28340	26712	Biodegradation
222	6693	355	34261	501	209329	334	27568	82.507	28386	26750	Biodegradation
228	6805	362	34414	498	208857	334	27609	82.724	28426	26793	Biodegradation
234	6917	371	34570	500	212816	337	27653	82.081	28478	26829	Biodegradation
240	7022	383	34717	502	217086	340	27695	81.392	28527	26862	Biodegradation
246	7143	390	34867	501	218076	341	27723	81.291	28558	26889	Biodegradation
252	7256	397	35012	502	220873	343	27756	80.870	28596	26916	Biodegradation
258	7362	405	35154	505	224457	346	27791	80.323	28638	26945	Biodegradation
264	7482	410	35290	501	223379	345	27808	80.565	28652	26963	Biodegradation

Table F-5 (Run-3) Data (O_2) for Determining Biodegradation from Individual Treatment of 1,000 mg/kg Propylene Glycol on Uncontaminated Soil

Time (hrs)	Mean Soil (uL)	Std Dev Soil	Mean PG1000 in Soil (uL)	Std Dev PG1000 in Soil	Pooled Estimator S_p^2	Standard Error	$X_{PG1000} - X_{soil}$	Calc T Value ($T_{crit} = 2.447$)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
270	7593	422	35428	504	228524	349	27835	79.731	28689	26981	Biodegradation
276	7688	432	35554	505	231944	352	27866	79.229	28727	27005	Biodegradation
282	7782	438	35678	504	233487	353	27895	79.050	28759	27032	Biodegradation
288	7881	448	35803	505	236631	355	27921	78.596	28791	27052	Biodegradation
294	7980	454	35926	507	239914	358	27946	78.126	28822	27071	Biodegradation
300	8081	462	36050	507	242584	360	27969	77.758	28849	27089	Biodegradation
306	8180	470	36171	511	247907	364	27992	76.981	28881	27102	Biodegradation
312	8277	482	36296	514	253534	368	28019	76.197	28919	27119	Biodegradation
318	8382	493	36419	513	256361	370	28038	75.826	28943	27133	Biodegradation
324	8476	500	36530	518	262094	374	28055	75.037	28969	27140	Biodegradation
330	8549	511	36630	522	268881	379	28081	74.155	29008	27155	Biodegradation
336	8624	514	36729	520	268144	378	28105	74.319	29030	27180	Biodegradation

Figure F-5 Difference Between the Means (O_2) and 95% CI for 1,000 mg/kg Propylene Glycol on Uncontaminated Soil



Appendix G: Statistical Procedures for Determining whether or not Measurable Biodegradation Occurred from the Combined ADF Chemicals (Propylene Glycol with Tolyltriazole) on Uncontaminated Soil

The data listed in the following five tables and figures explains the possible types of (decreased/no influence/increased) on biodegradation from a combination of chemical components (PG with TTA) on uncontaminated soil. This determination was made by comparing the O_2 consumption of the soil contaminated with both PG and TTA against the soil contaminated with PG only and TTA only. A two-sample t-test was performed using a significance level of $\alpha = 0.05$. A 95% CI was developed from the t-test results to depict the O_2 consumption effects. Both populations were assumed to be normal and the two population variances were assumed to be equal.

> H_o: There was no effect on the O_2 consumption due to combining the two contaminates
> H_a: There was an effect (decreased or increased) on the O_2 consumption due to the two contaminates

The pooled estimator, which was an estimate of the common population variance was determined by using the following equation (Devore, 358):

$$S_p^2 = \frac{(n_1-1)*S_1^2 + (n_2-1)*S_2^2 + (n_3-1)*S_3^2 + (n_4-1)*S_4^2}{(n_1+n_2+n_3+n_4) - 2}$$

Where n_1 through n_n are the sample sizes of the respective treatments, and S_1 through S_n are the standard deviations of the respective treatments.

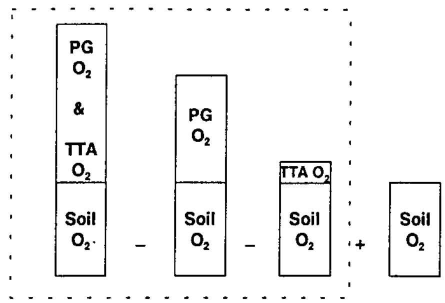
The standard error was determined by the following equations (Devore, 358):

$$\text{Std-Error} = S_p\,(1/n_1+1/n_2+1/n_3+1/n_4)^{1/2}$$

The calculated t-statistic (t) was then determined by dividing the difference of the means by the standard error.

$$t = \frac{(X_{PG\&TTA} - X_{TTA} - X_{PG}) + X_{soil}}{(\text{Std-Error})}$$

Shown below, is a visual depiction of the t-test and CI set-up with the O_2 mean totals.



This set-up provides a comparison of just the combined affects to be compared to the individual affects of ADF componts on soil.

The t-critical (t_{crit}) was determined for a <u>two-tailed test</u> since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis, thus $\alpha/2$ was used.

$$t_{crit} = t_{\alpha/2,\,(n1+n2+n3+n4)-2} = 2.201 \text{ (Devore, 707)}$$

Given: $\alpha = 0.05$
$n_1 = 3$ (number blank microcosms)
$n_2 = 5$ (number PG only microcosms)
$n_3 = 5$ (number TTA only microcosms)
$n_4 = 5$ (number PG & TTA microcosms)

The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical. An example of the test statistic is shown below:

$t \leq -t_{crit}$	$t \leq -2.201$	Inhibition
$t \geq t_{crit}$	$t \geq 2.201$	Biodegradation

The t-critical (t_{crit}) was determined for a two-tailed test since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical.

The upper and lower 95% confidence intervals were determined by using the following equation [Devore, 361]. This data was shown with the difference of the means (for the sample at its particular position on the time line) in Figures F-1 through F-4.

$$(X_{PG\&TTA} - X_{TTA} - X_{PG}) + X_{soil}) \pm (t_{\alpha/2,\,(n1+n2+n3+n4)-2}) * (S_p) * (1/n_1+1/n_2+1/n_3+1/n_4)^{1/2}$$

List of Tables

List of Figures

Table G-1 (Run-1) Data (O_2) for Determining Biodegradation of 25 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	Mean Blank Soil	Std Dev Blank Soil	Mean TTA25	Std Dev TTA25	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA25	Std Dev PG1000 TTA25	Pooled Estimator	Std Error	$X_{TTA25/PG1000}-X_{TTA25}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0	0	0	0	0.000	0	0	N/A
6	495	56	603	104	1993	83	1729	59	5687	73	-372	-5.103	-194	-550	Inhibition
12	818	72	1100	270	3716	160	3266	119	28867	164	-732	-4.458	-330	-1133	Inhibition
18	1075	79	1529	422	5719	269	5176	201	73538	262	-997	-3.804	-356	-1638	Inhibition
24	1309	82	1908	569	7695	436	7162	287	150087	374	-1132	-3.025	-216	-2048	Inhibition
30	1548	90	2263	730	9681	604	9141	374	260268	493	-1256	-2.547	-50	-2462	Inhibition
36	1769	101	2620	876	11320	765	10818	434	386542	601	-1353	-2.253	117	-2823	No effect
42	1926	112	2847	1002	12693	985	12194	486	554551	719	-1421	-1.976	339	-3182	No effect
48	2049	113	3047	1138	14181	1261	13658	545	797170	863	-1522	-1.764	589	-3632	No effect
54	2240	114	3349	1281	15874	1525	15343	633	1093298	1010	-1639	-1.623	832	-4111	No effect
60	2431	112	3645	1417	17539	1785	16975	716	1428456	1155	-1778	-1.540	1047	-4604	No effect
66	2619	113	3929	1556	19109	2066	18536	799	1834090	1308	-1884	-1.440	1318	-5085	No effect
72	2797	119	4212	1690	20651	2292	20037	865	2216207	1438	-2029	-1.411	1491	-5548	No effect
78	2986	126	4477	1819	22161	2439	21513	910	2522696	1534	-2140	-1.394	1615	-5894	No effect
84	3186	132	4774	1934	23588	2576	22972	941	2818298	1622	-2204	-1.359	1765	-6172	No effect
90	3362	138	5033	2040	24962	2679	24350	976	3074783	1694	-2283	-1.347	1863	-6428	No effect
96	3518	143	5259	2136	26249	2747	25696	1003	3281336	1750	-2294	-1.311	1988	-6576	No effect
102	3709	147	5547	2226	27464	2792	27021	1026	3453061	1795	-2280	-1.270	2113	-6673	No effect
108	3880	151	5779	2313	28636	2783	28271	1044	3548439	1820	-2264	-1.244	2189	-6717	No effect
114	4058	157	6015	2396	29718	2736	29424	1071	3597281	1832	-2250	-1.228	2234	-6734	No effect
120	4238	162	6239	2476	30656	2683	30504	1063	3618018	1838	-2154	-1.172	2342	-6651	No effect
126	4428	168	6477	2545	31507	2609	31458	1084	3618007	1838	-2098	-1.142	2399	-6595	No effect
132	4622	172	6719	2611	32242	2538	32297	1105	3623286	1839	-2042	-1.110	2458	-6542	No effect
138	4808	176	6940	2673	32840	2519	32996	1116	3688036	1855	-1976	-1.065	2564	-6516	No effect
144	4996	182	7165	2730	33356	2525	33581	1112	3770835	1876	-1945	-1.037	2645	-6536	No effect
150	5181	183	7389	2783	33812	2551	34056	1118	3879615	1903	-1964	-1.032	2692	-6621	No effect
156	5366	188	7612	2837	34202	2612	34483	1112	4031786	1940	-1965	-1.013	2782	-6712	No effect
162	5534	189	7808	2882	34546	2695	34859	1103	4201236	1980	-1962	-0.991	2884	-6807	No effect
168	5705	196	8013	2925	34868	2779	35215	1101	4378675	2022	-1961	-0.970	2986	-6908	No effect
174	5862	203	8204	2966	35169	2867	35541	1108	4567168	2065	-1970	-0.954	3082	-7023	No effect
180	6015	206	8379	3009	35458	2950	35850	1108	4751375	2106	-1972	-0.937	3181	-7125	No effect
186	6151	215	8531	3048	35734	3031	36140	1108	4931992	2146	-1975	-0.920	3275	-7225	No effect
192	6277	219	8700	3083	35979	3110	36412	1115	5111524	2184	-1990	-0.911	3355	-7335	No effect
198	6452	233	8928	3119	36271	3203	36742	1132	5324101	2229	-2005	-0.900	3449	-7460	No effect
204	6595	237	9103	3153	36541	3288	37023	1144	5523111	2270	-2025	-0.892	3531	-7581	No effect
210	6743	247	9271	3189	36806	3372	37310	1152	5725192	2312	-2024	-0.876	3632	-7681	No effect
216	6888	259	9461	3222	37120	3573	37600	1166	6135462	2393	-2093	-0.875	3763	-7949	No effect
222	7016	264	9595	3256	37374	3651	37845	1174	6335600	2432	-2108	-0.867	3842	-8058	No effect
228	7149	267	9755	3288	37613	3733	38092	1189	6548287	2472	-2127	-0.860	3923	-8176	No effect
234	7278	268	9891	3314	37841	3807	38327	1201	6737777	2508	-2127	-0.848	4009	-8264	No effect

Table G-1 (Run-1) Data (O_2) for Determining Biodegradation of 25 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	Mean Blank Soil	Std Dev Blank Soil	Mean TTA25	Std Dev TTA25	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA25	Std Dev PG1000 TTA25	Pooled Estimator	Std Error	$X_{TTA25/PG1000}-X_{TTA25}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
240	7404	272	10034	3341	38057	3874	38557	1203	6914965	2540	-2129	-0.838	4087	-8346	No effect
246	7517	276	10157	3369	38267	3941	38776	1208	7096610	2574	-2131	-0.828	4167	-8428	No effect
252	7627	282	10271	3398	38469	4006	38981	1215	7276666	2606	-2132	-0.818	4245	-8510	No effect
258	7739	286	10374	3424	38664	4067	39175	1223	7450559	2637	-2123	-0.805	4329	-8576	No effect
264	7849	288	10508	3447	38850	4130	39382	1228	7622439	2667	-2127	-0.797	4400	-8654	No effect
270	7962	297	10619	3475	39038	4184	39583	1238	7788689	2696	-2112	-0.783	4485	-8710	No effect
276	8066	308	10721	3500	39221	4236	39773	1244	7947120	2723	-2103	-0.772	4561	-8768	No effect
282	8166	314	10819	3527	39388	4281	39946	1257	8097538	2749	-2094	-0.762	4633	-8822	No effect
288	8264	320	10922	3551	39551	4325	40125	1261	8238222	2773	-2084	-0.751	4702	-8869	No effect
294	8359	331	11016	3575	39705	4352	40290	1271	8346589	2791	-2072	-0.742	4758	-8902	No effect
300	8462	337	11117	3598	39855	4372	40466	1281	8440227	2807	-2044	-0.728	4824	-8912	No effect
306	8555	343	11207	3622	39992	4386	40622	1291	8521104	2820	-2023	-0.717	4877	-8924	No effect
312	8643	355	11302	3645	40125	4399	40782	1295	8594021	2832	-2002	-0.707	4929	-8932	No effect
318	8727	360	11386	3669	40252	4412	40925	1306	8672948	2845	-1986	-0.698	4976	-8948	No effect
324	8820	370	11501	3691	40387	4424	41092	1318	8749728	2858	-1977	-0.692	5016	-8969	No effect
330	8910	373	11598	3712	40512	4435	41249	1321	8814134	2868	-1952	-0.680	5067	-8970	No effect
336	8992	373	11684	3732	40628	4447	41397	1326	8882142	2879	-1923	-0.668	5123	-8968	No effect

Figure G-1 Difference Between the Means (O_2) and 95% CI for the Linear Combination of 25 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

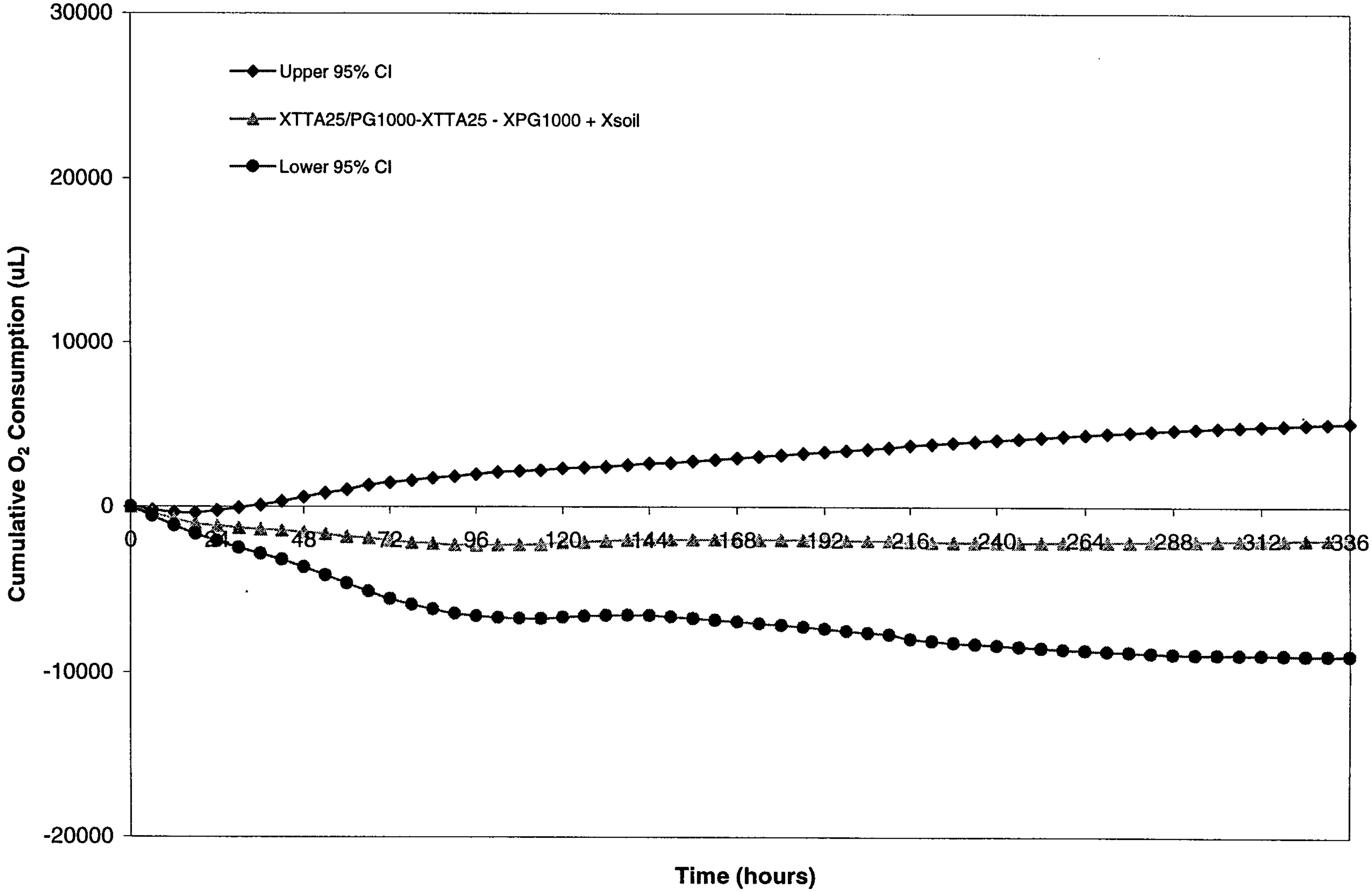


Table G-2 (Run-2) Data (O_2) for Determining Biodegradation of 250 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	Mean Blank Soil	Std Dev Blank Soil	Mean TTA250	Std Dev TTA250	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA250	Std Dev PG1000 TTA250	Pooled Estimator	Std Error	$X_{TTA250/PG1000}-X_{TTA250}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0	0	0	0	0.000	0	0	N/A
6	960	90	907	80	2305	66	1712	56	4504	65	-540	-8.328	-381	-699	Inhibition
12	1502	146	1470	135	3927	150	2906	125	16778	125	-988	-7.895	-682	-1294	Inhibition
18	1894	201	1877	185	5793	260	4026	199	40347	194	-1750	-9.016	-1275	-2225	Inhibition
24	2180	243	2188	225	8268	423	5155	276	83894	280	-3121	-11.155	-2437	-3806	Inhibition
30	2414	268	2453	258	11042	588	6351	359	144347	367	-4729	-12.885	-3831	-5628	Inhibition
36	2649	285	2726	291	13788	758	7742	458	227353	461	-6123	-13.292	-4996	-7250	Inhibition
42	2857	292	2993	322	16399	812	9335	525	270528	502	-7199	-14.327	-5970	-8429	Inhibition
48	3038	296	3232	352	18855	861	11116	584	312365	540	-7933	-14.692	-6612	-9254	Inhibition
54	3196	296	3424	377	21017	970	13036	676	395807	608	-8210	-13.508	-6723	-9698	Inhibition
60	3347	297	3642	403	23139	995	15330	784	452965	650	-8104	-12.463	-6513	-9695	Inhibition
66	3483	300	3818	425	25087	1014	17800	893	512822	692	-7622	-11.017	-5929	-9315	Inhibition
72	3632	298	4002	438	26824	1109	20396	1023	628023	766	-6798	-8.880	-4925	-8672	Inhibition
78	3817	294	4237	461	28328	1086	23037	1109	666236	789	-5711	-7.242	-3781	-7641	Inhibition
84	4006	294	4468	482	29458	988	25646	1238	696023	806	-4274	-5.302	-2301	-6246	Inhibition
90	4198	291	4697	492	30248	919	28150	1347	736366	829	-2597	-3.133	-569	-4626	Inhibition
96	4382	285	4923	513	30848	839	30552	1460	784919	856	-837	-0.977	1258	-2931	Inhibition
102	4557	279	5140	529	31305	803	32804	1533	828478	879	916	1.042	3068	-1236	Inhibition
108	4700	273	5321	547	31644	806	34805	1582	872513	902	2540	2.815	4748	332	Inhibition
114	4843	269	5493	563	31930	820	36615	1609	903681	918	4034	4.393	6282	1787	Inhibition
120	4963	258	5625	577	32177	832	38194	1625	924820	929	5355	5.763	7628	3081	Inhibition
126	5090	250	5780	589	32417	842	39489	1657	958003	946	6381	6.748	8695	4067	Inhibition
132	5210	244	5922	598	32645	840	40623	1684	982689	958	7266	7.587	9610	4923	No effect
138	5334	236	6066	610	32869	840	41549	1716	1012365	972	7948	8.177	10327	5569	No effect
144	5448	230	6200	617	33058	845	42257	1729	1027290	979	8447	8.627	10843	6051	No effect
150	5548	220	6311	630	33250	844	42859	1729	1031009	981	8846	9.018	11247	6446	Biodegradation
156	5652	211	6439	635	33447	843	43413	1720	1023908	978	9179	9.390	11571	6787	Biodegradation
162	5762	202	6551	642	33644	841	43885	1719	1023777	978	9453	9.670	11844	7061	Biodegradation
168	5875	192	6682	647	33833	839	44309	1716	1021743	977	9668	9.900	12058	7278	Biodegradation
174	5960	185	6789	655	33984	838	44627	1719	1026043	979	9814	10.028	12208	7419	Biodegradation
180	6038	176	6882	659	34131	841	44917	1719	1028119	980	9942	10.149	12339	7545	Biodegradation
186	6117	163	6973	665	34271	844	45183	1727	1037828	984	10056	10.217	12464	7648	Biodegradation
192	6188	155	7055	666	34402	840	45431	1733	1041320	986	10162	10.308	12574	7749	Biodegradation
198	6251	148	7141	675	34525	839	45667	1739	1048450	989	10252	10.364	12673	7832	Biodegradation
204	6324	140	7221	681	34655	836	45895	1749	1058176	994	10342	10.407	12774	7911	Biodegradation
210	6389	131	7300	691	34782	834	46104	1756	1066687	998	10412	10.435	12853	7970	Biodegradation
216	6459	124	7370	696	34903	835	46306	1767	1077754	1003	10492	10.462	12947	8038	Biodegradation
222	6532	116	7450	703	35028	832	46515	1773	1084184	1006	10569	10.507	13031	8108	Biodegradation
228	6601	110	7531	711	35148	831	46717	1780	1092477	1010	10639	10.536	13109	8168	Biodegradation
234	6669	105	7608	719	35265	831	46913	1786	1100491	1013	10710	10.568	13190	8230	Biodegradation

Table G-2 (Run-2) Data (O_2) for Determining Biodegradation of 250 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	Mean Blank Soil	Std Dev Blank Soil	Mean TTA250	Std Dev TTA250	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA250	Std Dev PG1000 TTA250	Pooled Estimator	Std Error	$X_{TTA250/PG1000}-X_{TTA250}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
240	6736	97	7688	728	35380	829	47105	1792	1108312	1017	10774	10.593	13262	8285	Biodegradation
246	6800	90	7758	735	35494	829	47286	1798	1116418	1021	10834	10.613	13332	8336	Biodegradation
252	6864	84	7832	743	35604	832	47467	1807	1127960	1026	10895	10.619	13406	8384	Biodegradation
258	6921	81	7895	751	35704	830	47632	1812	1134938	1029	10953	10.642	13471	8434	Biodegradation
264	6987	78	7964	758	35810	828	47799	1820	1143847	1033	11012	10.658	13540	8483	Biodegradation
270	7053	74	8036	766	35913	830	47965	1825	1152600	1037	11069	10.672	13607	8531	Biodegradation
276	7114	73	8108	770	36012	830	48123	1832	1160619	1041	11117	10.681	13664	8570	Biodegradation
282	7176	65	8175	777	36112	832	48282	1838	1169573	1045	11171	10.692	13727	8614	Biodegradation
288	7232	61	8236	786	36205	834	48431	1846	1180916	1050	11222	10.689	13791	8653	Biodegradation
294	7284	56	8296	795	36295	833	48568	1852	1188901	1053	11262	10.691	13839	8684	Biodegradation
300	7336	53	8350	802	36380	833	48705	1858	1197402	1057	11311	10.700	13898	8724	Biodegradation
306	7382	49	8398	811	36459	835	48835	1865	1208573	1062	11360	10.696	13958	8761	Biodegradation
312	7439	44	8462	817	36546	836	48977	1871	1217230	1066	11406	10.701	14014	8798	Biodegradation
318	7494	43	8525	823	36629	842	49113	1879	1228885	1071	11454	10.695	14074	8833	Biodegradation
324	7549	47	8597	829	36712	844	49254	1884	1238015	1075	11493	10.692	14124	8863	Biodegradation
330	7604	49	8658	837	36790	848	49387	1891	1249281	1080	11543	10.690	14185	8901	Biodegradation
336	7654	53	8719	843	36870	847	49517	1898	1257653	1083	11582	10.691	14234	8931	Biodegradation

Figure G-2 Difference Between the Means (O_2) and 95% CI for the Linear Combination of 250 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

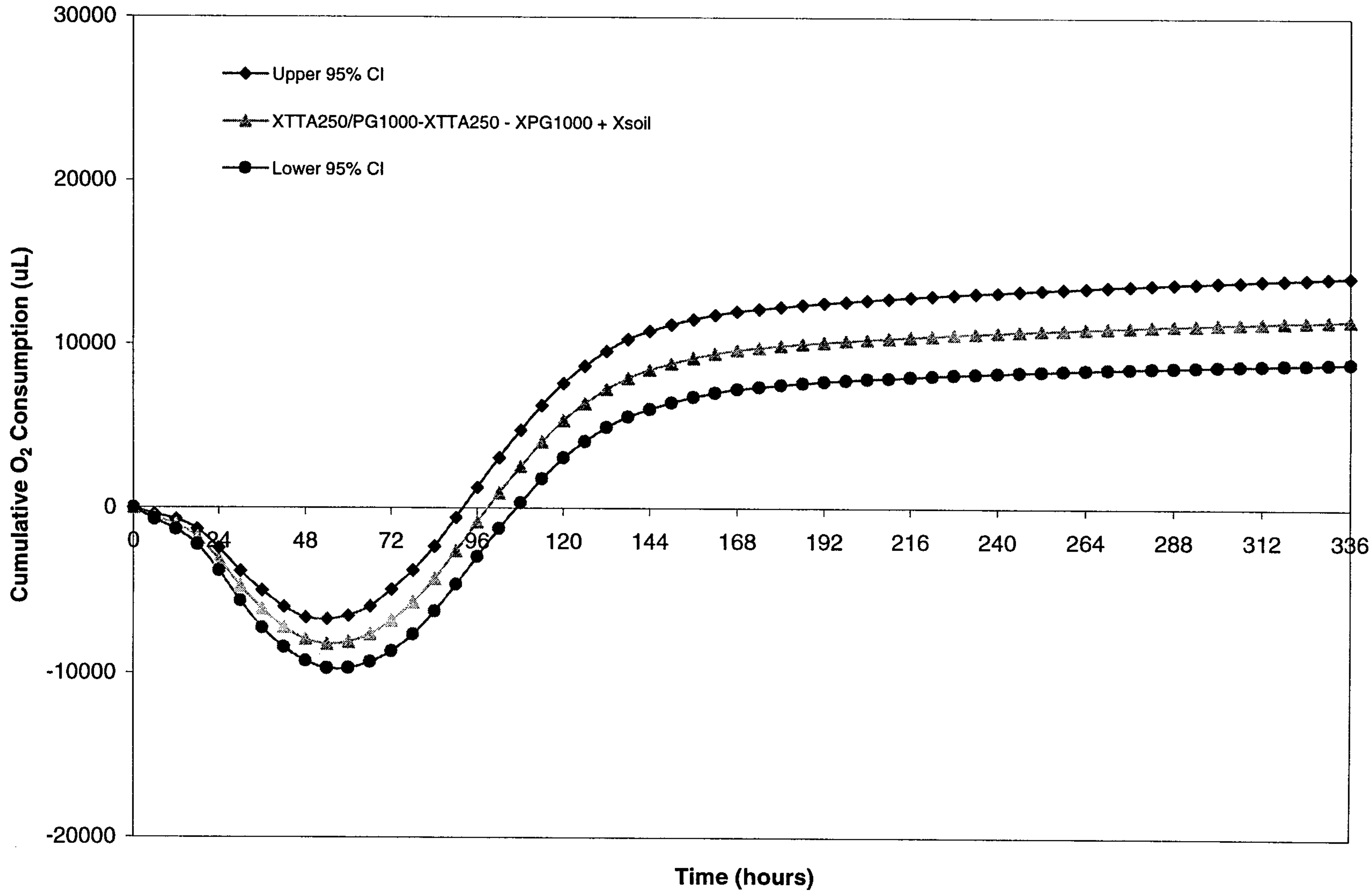


Table G-3 (Run-3) Data (O_2) for Determining Biodegradation of 500 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	Mean Blank Soil	Std Dev Blank Soil	Mean TTA500	Std Dev TTA500	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA500	Std Dev PG1000 TTA500	Pooled Estimator	Std Error	$X_{TTA500/PG1000}-X_{TTA500}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0	0	0	0	0.000	0	0	N/A
6	610	25	683	37	2189	61	1188	20	1462	37	-1074	-29.079	-984	-1165	Inhibition
12	981	29	1147	84	3944	124	2078	28	5945	74	-2032	-27.275	-1849	-2214	Inhibition
18	1258	34	1563	123	6240	233	2941	49	18087	130	-3604	-27.740	-3286	-3922	Inhibition
24	1493	36	1940	158	8994	354	3831	67	38788	190	-5610	-29.485	-5144	-6076	Inhibition
30	1725	43	2269	189	11593	452	4763	92	62370	241	-7374	-30.563	-6784	-7964	Inhibition
36	1949	48	2582	220	14043	539	5762	121	88745	288	-8915	-30.976	-8211	-9619	Inhibition
42	2169	35	2887	247	16312	568	6816	140	101086	307	-10214	-33.253	-9462	-10966	Inhibition
48	2361	34	3161	271	18520	622	7930	174	122636	338	-11389	-33.664	-10561	-12217	Inhibition
54	2544	40	3394	295	20489	638	9066	204	133878	353	-12273	-34.719	-11408	-13138	Inhibition
60	2718	40	3631	316	22295	624	10301	194	131958	351	-12907	-36.779	-12048	-13766	Inhibition
66	2878	48	3826	337	23960	630	11633	273	146671	370	-13274	-35.877	-12369	-14180	Inhibition
72	3032	60	4053	359	25442	630	13085	370	165981	394	-13378	-33.988	-12415	-14341	Inhibition
78	3197	70	4232	372	26650	571	14591	493	177467	407	-13094	-32.173	-12098	-14090	Inhibition
84	3357	79	4417	391	27646	513	16209	637	206245	439	-12498	-28.486	-11424	-13571	Inhibition
90	3519	92	4590	409	28411	456	17860	794	252425	485	-11621	-23.942	-10433	-12809	Inhibition
96	3673	113	4779	424	28994	421	19597	958	320591	547	-10503	-19.200	-9164	-11841	Inhibition
102	3823	123	4957	442	29441	398	21351	1135	412756	621	-9224	-14.861	-7705	-10743	Inhibition
108	3968	134	5117	457	29819	407	23134	1322	532435	705	-7834	-11.113	-6109	-9559	Inhibition
114	4060	140	5210	467	30113	415	24870	1488	653730	781	-6393	-8.185	-4482	-8305	Inhibition
120	4231	153	5428	485	30413	414	26718	1658	791772	860	-4892	-5.690	-2788	-6995	Inhibition
126	4387	165	5630	498	30696	431	28678	1845	963231	948	-3260	-3.439	-940	-5581	Inhibition
132	4566	177	5850	514	30983	444	30698	2002	1120843	1023	-1570	-1.535	933	-4072	no effect
138	4730	188	6053	536	31253	445	32582	2127	1257074	1083	5	0.005	2656	-2645	no effect
144	4888	204	6256	549	31524	460	34406	2211	1356005	1125	1514	1.346	4267	-1238	no effect
150	5068	219	6468	568	31806	470	36153	2266	1425743	1154	2947	2.555	5770	124	Biodegradation
156	5226	229	6641	583	32059	466	37776	2284	1449373	1163	4302	3.699	7148	1456	Biodegradation
162	5388	248	6822	599	32318	477	39284	2266	1438670	1159	5533	4.775	8369	2697	Biodegradation
168	5532	262	6996	615	32553	478	40601	2261	1438441	1159	6585	5.683	9420	3750	Biodegradation
174	5677	280	7150	630	32777	475	41825	2222	1399470	1143	7575	6.628	10371	4778	Biodegradation
180	5813	290	7290	641	32992	482	42915	2177	1356271	1125	8445	7.506	11199	5692	Biodegradation
186	5958	301	7448	654	33204	489	43872	2140	1323485	1111	9178	8.258	11898	6459	Biodegradation
192	6081	308	7572	667	33389	487	44714	2089	1272981	1090	9833	9.021	12501	7166	Biodegradation
198	6208	317	7704	679	33576	493	45451	2050	1239562	1076	10379	9.649	13011	7747	Biodegradation
204	6323	328	7817	694	33754	497	46116	1996	1191196	1054	10868	10.307	13448	8288	Biodegradation
210	6436	339	7929	705	33919	495	46694	1948	1148786	1035	11282	10.896	13816	8748	Biodegradation
216	6567	348	8081	717	34093	501	47248	1901	1109695	1018	11640	11.437	14130	9149	Biodegradation
222	6693	355	8213	724	34261	501	47744	1849	1064429	997	11964	12.003	14403	9525	Biodegradation
228	6805	362	8329	733	34414	498	48173	1813	1034619	983	12234	12.450	14639	9830	Biodegradation
234	6917	371	8438	745	34570	500	48567	1776	1007282	970	12476	12.867	14849	10104	Biodegradation

Table G-3 (Run-3) Data (O_2) for Determining Biodegradation of 500 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	Mean Blank Soil	Std Dev Blank Soil	Mean TTA500	Std Dev TTA500	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA500	Std Dev PG1000 TTA500	Pooled Estimator	Std Error	$X_{TTA500/PG1000}-X_{TTA500}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
240	7022	383	8570	758	34717	502	48938	1738	980021	956	12673	13.251	15014	10333	Biodegradation
246	7143	390	8679	772	34867	501	49286	1714	964946	949	12883	13.575	15205	10561	Biodegradation
252	7256	397	8806	779	35012	502	49610	1690	948931	941	13048	13.864	15351	10745	Biodegradation
258	7362	405	8923	792	35154	505	49905	1673	941021	937	13191	14.076	15484	10898	Biodegradation
264	7482	410	9065	802	35290	501	50203	1662	934917	934	13331	14.271	15616	11045	Biodegradation
270	7593	422	9189	812	35428	504	50477	1655	935146	934	13453	14.400	15739	11167	Biodegradation
276	7688	432	9294	823	35554	505	50722	1653	939296	936	13562	14.484	15853	11271	Biodegradation
282	7782	438	9379	833	35678	504	50945	1653	944550	939	13670	14.560	15968	11373	Biodegradation
288	7881	448	9497	845	35803	505	51176	1653	950329	942	13758	14.608	16062	11453	Biodegradation
294	7980	454	9612	855	35926	507	51396	1660	961380	947	13838	14.608	16156	11520	Biodegradation
300	8081	462	9725	862	36050	507	51619	1665	969652	951	13925	14.638	16253	11597	Biodegradation
306	8180	470	9837	869	36171	511	51832	1671	979557	956	14003	14.645	16343	11664	Biodegradation
312	8277	482	9949	882	36296	514	52041	1681	996554	964	14073	14.592	16433	11713	Biodegradation
318	8382	493	10065	889	36419	513	52248	1689	1006670	969	14146	14.594	16518	11774	Biodegradation
324	8476	500	10172	899	36530	518	52441	1694	1017801	975	14215	14.584	16600	11830	Biodegradation
330	8549	511	10279	906	36630	522	52611	1707	1034483	983	14251	14.503	16655	11846	Biodegradation
336	8624	514	10380	912	36729	520	52776	1716	1044643	987	14291	14.473	16707	11875	Biodegradation

Figure G-3 Difference Between the Means (O_2) and 95% CI for the Linear Combination of 500 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

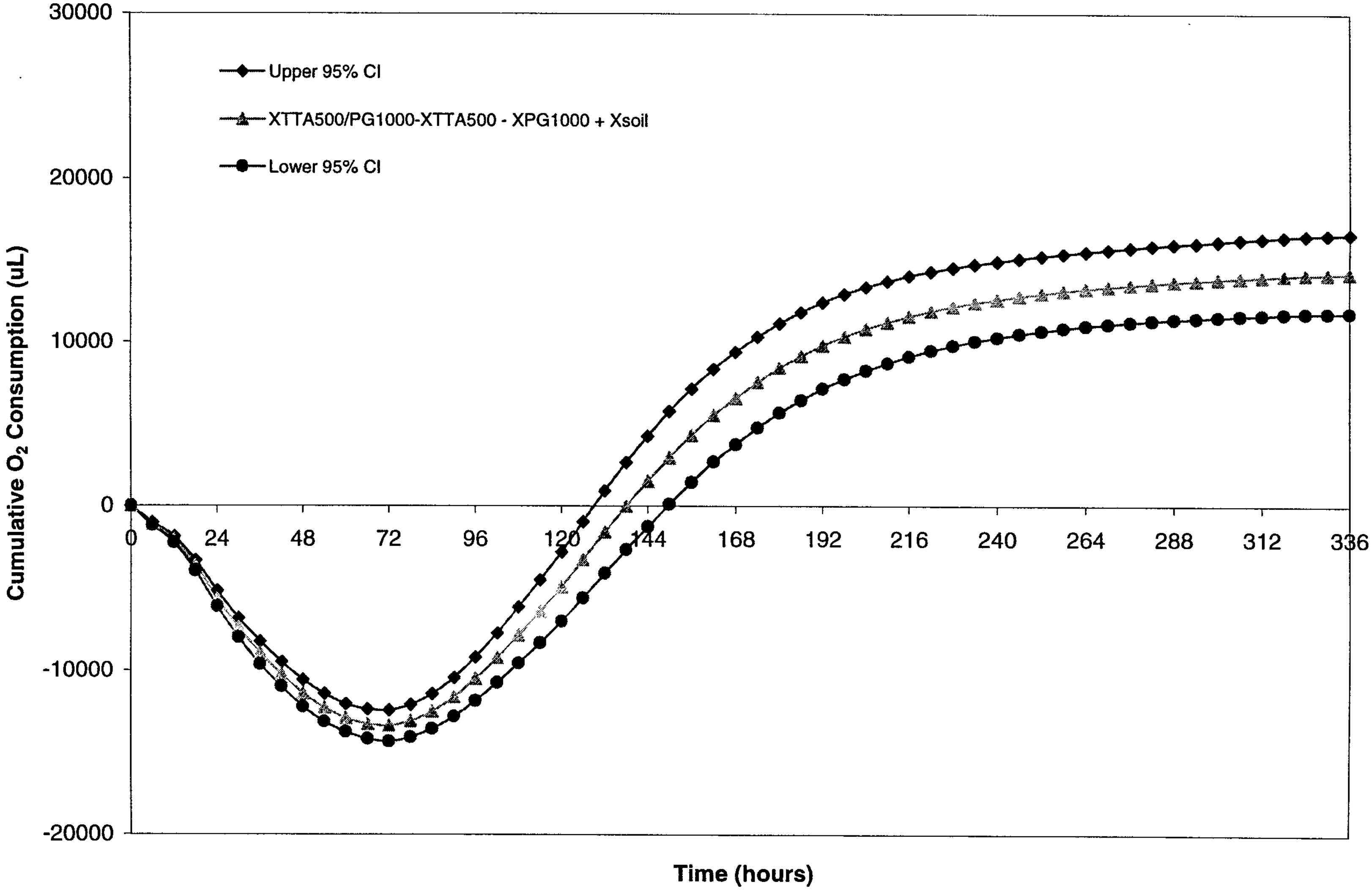


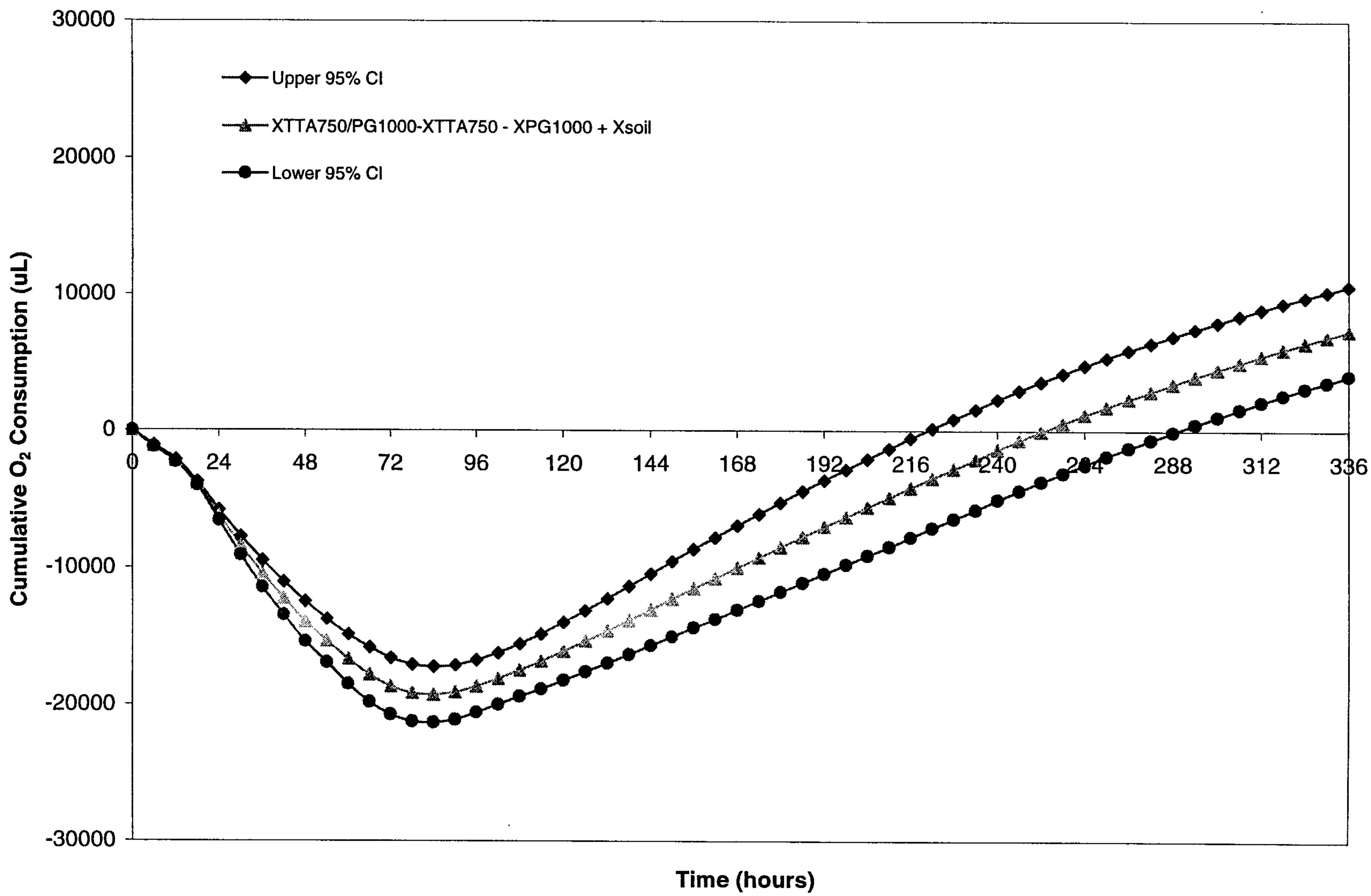
Table G-4 (Run-5) Data (O_2) for Determining Biodegradation of 750 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	(Run-3) Mean Blank Soil	(Run-3) Std Dev Blank Soil	Mean TTA750	Std Dev TTA750	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA750	Std Dev PG1000 TTA750	Pooled Estimator	Std Error	$X_{TTA750/PG1000}-X_{TTA750}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
0	0	0	0	0	0	0	0	0	0	0	0	0.000	0	0	N/A
6	610	25	553	14	2161	53	948	4	843	28	-1156	-41.222	-1088	-1225	Inhibition
12	981	29	950	24	3861	55	1615	32	1276	35	-2215	-64.179	-2131	-2299	Inhibition
18	1258	34	1306	44	6060	82	2224	65	3340	56	-3884	-69.571	-3748	-4021	Inhibition
24	1493	36	1608	60	8891	306	2800	93	26614	158	-6206	-39.377	-5820	-6592	Inhibition
30	1725	43	1913	77	11652	565	3418	127	85445	282	-8422	-29.822	-7731	-9113	Inhibition
36	1949	48	2212	93	14282	812	4080	167	174234	403	-10464	-25.949	-9477	-11451	Inhibition
42	2169	35	2505	106	16693	997	4782	156	257345	490	-12247	-24.989	-11048	-13446	Inhibition
48	2361	34	2796	110	19025	1216	5537	196	382317	597	-13922	-23.306	-12460	-15383	Inhibition
54	2544	40	3075	118	21152	1328	6346	213	455608	652	-15337	-23.519	-13741	-16933	Inhibition
60	2718	40	3351	124	23241	1514	7199	247	592736	744	-16674	-22.418	-14854	-18494	Inhibition
66	2878	48	3613	134	25171	1666	8088	286	719315	819	-17818	-21.746	-15813	-19823	Inhibition
72	3032	60	3864	140	26840	1712	9002	347	768038	847	-18670	-22.051	-16598	-20741	Inhibition
78	3197	70	4141	142	28208	1704	9980	430	777937	852	-19173	-22.500	-17087	-21258	Inhibition
84	3357	79	4395	150	29246	1653	10999	506	753096	838	-19285	-23.003	-17234	-21337	Inhibition
90	3519	92	4658	156	30018	1569	12044	595	711219	815	-19113	-23.459	-17119	-21107	Inhibition
96	3673	113	4877	158	30556	1452	13091	694	655358	782	-18669	-23.870	-16755	-20583	Inhibition
102	3823	123	5098	160	30981	1373	14138	802	640251	773	-18118	-23.438	-16227	-20010	Inhibition
108	3968	134	5329	170	31356	1342	15230	916	669375	790	-17488	-22.125	-15554	-19422	Inhibition
114	4060	140	5531	178	31685	1338	16301	1034	725183	823	-16855	-20.488	-14842	-18868	Inhibition
120	4231	153	5739	183	31985	1350	17384	1161	803378	866	-16109	-18.604	-13990	-18228	Inhibition
126	4387	165	5910	191	32264	1364	18418	1288	892279	913	-15369	-16.841	-13135	-17602	Inhibition
132	4566	177	6115	203	32542	1381	19485	1418	993476	963	-14605	-15.167	-12249	-16962	Inhibition
138	4730	188	6311	217	32809	1395	20535	1548	1101986	1014	-13855	-13.661	-11373	-16336	Inhibition
144	4888	204	6475	228	33048	1411	21559	1675	1217504	1066	-13076	-12.266	-10467	-15684	Inhibition
150	5068	219	6639	241	33290	1429	22568	1797	1337889	1117	-12293	-11.001	-9558	-15027	Inhibition
156	5226	229	6816	255	33534	1445	23602	1914	1460901	1168	-11522	-9.867	-8665	-14379	Inhibition
162	5388	248	7030	272	33793	1460	24646	2024	1582961	1215	-10789	-8.876	-7815	-13764	Inhibition
168	5532	262	7195	283	34008	1475	25653	2117	1693571	1257	-10019	-7.969	-6942	-13095	Inhibition
174	5677	280	7373	295	34237	1491	26656	2204	1801421	1297	-9278	-7.155	-6105	-12451	Inhibition
180	5813	290	7530	305	34451	1505	27644	2284	1904293	1333	-8524	-6.394	-5262	-11787	Inhibition
186	5958	301	7668	312	34650	1522	28587	2354	1999667	1366	-7773	-5.690	-4430	-11116	Inhibition
192	6081	308	7793	320	34829	1536	29509	2416	2086154	1395	-7033	-5.040	-3618	-10447	Inhibition
198	6208	317	7929	329	35013	1548	30419	2470	2164860	1421	-6315	-4.442	-2836	-9793	Inhibition
204	6323	328	8068	340	35194	1561	31338	2516	2234335	1444	-5601	-3.879	-2068	-9135	Inhibition
210	6436	339	8212	350	35381	1574	32256	2553	2293905	1463	-4901	-3.349	-1320	-8481	Inhibition
216	6567	348	8352	361	35562	1587	33171	2583	2344422	1479	-4176	-2.823	-557	-7796	Inhibition
222	6693	355	8493	372	35745	1601	34070	2601	2382887	1491	-3476	-2.331	174	-7125	No effect
228	6805	362	8641	383	35924	1614	34963	2612	2410516	1500	-2797	-1.865	873	-6467	No effect
234	6917	371	8761	393	36088	1629	35817	2615	2428717	1506	-2114	-1.404	1570	-5798	No effect

Table G-4 (Run-5) Data (O_2) for Determining Biodegradation of 750 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

Time (hours)	(Run-3) Mean Blank Soil	(Run-3) Std Dev Blank Soil	Mean TTA750	Std Dev TTA750	Mean PG1000	Std Dev PG1000	Mean PG1000 TTA750	Std Dev PG1000 TTA750	Pooled Estimator	Std Error	$X_{TTA750/PG1000}-X_{TTA750}-X_{PG1000}+X_{soil}$	Calc T Value (Tcrit = 2.447)	Upper 95% CI	Lower 95% CI	Biodegradation /Inhibition/ No effect
240	7022	383	8752	393	36205	1648	36561	2603	2429474	1506	-1374	-0.912	2311	-5059	No effect
246	7143	390	8849	399	36358	1663	37344	2591	2428429	1506	-720	-0.478	2964	-4404	No effect
252	7256	397	8927	403	36496	1674	38108	2575	2419001	1503	-59	-0.040	3617	-3736	No effect
258	7362	405	9029	419	36636	1685	38861	2551	2401455	1497	558	0.372	4221	-3106	No effect
264	7482	410	9140	426	36771	1694	39606	2525	2377427	1490	1177	0.790	4822	-2468	No effect
270	7593	422	9257	435	36910	1705	40335	2495	2352995	1482	1761	1.188	5387	-1866	No effect
276	7688	432	9344	442	37023	1713	41030	2460	2318581	1471	2351	1.598	5950	-1249	No effect
282	7782	438	9452	449	37148	1720	41717	2423	2281777	1459	2899	1.986	6470	-672	No effect
288	7881	448	9537	455	37265	1729	42382	2382	2241816	1446	3461	2.393	7001	-79	No effect
294	7980	454	9635	461	37381	1735	43032	2342	2202705	1434	3995	2.786	7504	487	Biodegradation
300	8081	462	9737	470	37494	1742	43675	2302	2164838	1421	4525	3.184	8004	1047	Biodegradation
306	8180	470	9835	477	37595	1747	44285	2260	2124740	1408	5036	3.576	8482	1590	Biodegradation
312	8277	482	9920	484	37702	1756	44890	2221	2091163	1397	5546	3.970	8965	2127	Biodegradation
318	8382	493	10032	491	37819	1761	45497	2183	2057945	1386	6027	4.349	9419	2636	Biodegradation
324	8476	500	10126	498	37925	1768	46079	2146	2025599	1375	6505	4.731	9869	3140	Biodegradation
330	8549	511	10215	504	38020	1773	46631	2113	1998211	1366	6945	5.086	10287	3603	Biodegradation
336	8624	514	10290	512	38109	1780	47169	2080	1972926	1357	7393	5.448	10714	4073	Biodegradation

Figure G-4 Difference Between the Means (O_2) and 95% CI for the Linear Combination of 750 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol



Appendix H: HPLC Analysis of Residual Tolyltriazole

HPLC analysis was performed on the amount of tolyltriazole residual available "before" and "after" respirometry analysis. HPLC analysis was performed for two concentration levels of tolyltriazole (25 – 250 mg/kg) within the soil. Tolyltriazole extracted/residuals had numerous pathways of degradation (biotic, absorption, chemical) before and after the respirometry experiments in soil.

Prior to analyses of tolyltriazole residuals in the soil environment, a calibration curve was established for HPLC analysis of tolyltriazole in a pure methanol (see Figure 3-2). Appendix C contains data and calculations. A subtle change in the specific gravity of extraction solutions occurred due to the additional H_2O from the soil mixing with the methanol used for extracting tolyltriazole from soil. This change in the specific gravity was accounted for in the conversion of tolyltriazole residuals using the calibration curve equation.

A step-by-step process for determining the potential degradation/residual tolyltriazole in soil was performed on the soil treated with ADF components "before " respirometry runs, in Table H-1 through Table H-4. Table H-1 contains the data for determining the soils moisture.

Table H-1
Calculations of Soil Moisture (Before Respirometry Experiments)

	Aluminum Container						
	Wt of Alum Cont (gm)	Wt of Alum Cont & Wet Soil (gm)	Wt of Alum Cont & Dry Soil (gm)	Wt of Wet Soil (gm)	Wt of Dry Soil (gm)	Wt of H_20 (gm)	% H_2O in Spent Soil
TTA_{25}	1.5540	18.2035	15.7225	16.6495	14.1685	2.4810	14.90%
TTA_{250}	1.5499	17.1490	14.8478	15.5991	13.2979	2.3012	14.75%
TTA_{500}	1.5530	14.9002	13.0375	13.3472	11.4845	1.8627	13.96%
PG_{1000} & TTA_{25}	1.5424	18.3540	15.9437	16.8116	14.4013	2.4103	14.34%
PG_{1000} & TTA_{250}	1.5462	18.8703	16.2536	17.3242	14.7074	2.6168	15.10%
PG_{1000} & TTA_{500}	1.5616	13.9762	12.1161	12.4146	10.5545	1.8601	14.98%

The measurements of soil moisture were determined through stand alone weight measurements of the soil media (see "Aluminum Container" section in the above data). A sample of the "wet" soil was weighed and measured, then dried at 85°C for 24 hrs, to obtain a "dry" soil sample. The weight of water removed from the soil was then calculated.

Table H-2 contains the weight measurements of the vials, methanol, and soil used in the HPLC analysis of "before" respirometry analysis (without biodegradation potential in soil). This was recalculated to determine the specific gravity of the mixture of methanol and H_2O (from moisture content determined in Table H-1) used to extract the tolyltriazole.

Table H-2
Weights used in Removal Efficiency (Before Respirometry Experiments)

	40 mL EPA Vial for HPLC Extraction Methods				
	Wt of 40 mL Vial (gm)	Wt Vial & Soil (gm)	Wt Vial & Soil & Methanol (gm)	Wt of Methanol in the Vial (gm)	Wt of Soil in the Vial (gm)
TTA_{25}	22.3196	34.8771	46.2010	11.3239	12.5575
TTA_{250}	22.4522	34.7269	46.2699	11.5429	12.2747
TTA_{500}	22.4064	37.3024	48.8810	11.5786	14.8959
PG_{1000} & TTA_{25}	22.4317	36.0177	47.6961	11.6785	13.5860
PG_{1000} & TTA_{250}	22.4771	34.0338	45.5804	11.5466	11.5567
PG_{1000} & TTA_{500}	22.3717	34.9987	46.6901	11.6914	12.6270

Note: Upon inoculation and mixing of the soil with the chemical solution, immediate extraction was performed. This allowed the assumption of minimal biodegradation. The biodegradation was considered negligible since anaerobic conditions were introduced with the sealed vials and little oxygen due to the filled vial volume with aqueous solution. Photodegradation was assumed negligible by the use of amber color vials.

Table H-3 contains the HPLC detection area (mAu*s) values for tolyltriazole residuals "before" respirometry (without biodegradation potential in soil).

Table H-3
HPLC Detection Areas for Tolyltriazole Residuals (Before Respirometry Experiments)

	Soil Extracted Solution (Meth+ H_20 + Toly) (mAu*s)	
	Average	Std Dev
TTA_{25}	173.98	2.36
TTA_{250}	1522.90	5.50
TTA_{500}	3811.32	3.14
PG_{1000} & TTA_{25}	177.61	2.14
PG_{1000} & TTA_{250}	1519.16	2.72
PG_{1000} & TTA_{500}	3265.56	4.11

Note: Each HPLC detection area listed above represents three measurements averaged.

The preliminary information was now gathered on soil moisture, mass of vials/methanol/soil, and the detection areas associated with the "before" respirometry soil treatments. This allowed the calculations of residual tolyltriazole from interaction with soil shown in the following steps of calculations in Table H-4 shown below:

Table H-4
Steps/Calculations for the Recovery Percentage of Tolyltriazole Residuals
(Before Respirometry Experiments)

	1		2	3	4	5	6	7	8
	HPLC Area (mAu*s)		Conc Conversion	Density of Methanol/H_2O mix in Vial	Mass of Toly in Vial	Soil in Vial	End Conc	Initial Conc	% recovered	
	Avg	Std Dev	x = y/9.01 (mg/L)	(wtH_2O+wtMeth) / (volH_2O+volMeth) (mg/mL)	[(conc/density)*(wt H20 + Meth)]/1000mL (mg toly)	(mg)	mg toly / kg soil	mg toly / kg soil	(End Conc/Initial Conc)*100 Avg	Std Dev
TTA_{25}	173.978	2.362	19.309	0.813	0.313	12.557	24.947	25.000	99.79%	1.35%
TTA_{250}	1522.904	5.504	169.024	0.812	2.779	12.275	226.389	250.000	90.56%	0.33%
TTA_{500}	3811.318	3.144	423.010	0.815	7.087	14.896	475.772	500.000	95.15%	0.08%
PG_{1000} & TTA_{25}	177.608	2.135	19.712	0.814	0.330	13.586	24.302	25.000	97.21%	1.17%
PG_{1000} & TTA_{250}	1519.161	2.725	168.608	0.811	2.762	11.557	238.980	250.000	95.59%	0.17%
PG_{1000} & TTA_{500}	3265.557	4.107	362.437	0.813	6.056	12.627	479.632	500.000	95.93%	0.12%

Step 1 The areas from HPLC analysis are listed in this step (y = areas). The equation (y = 9.01x) was derived in section 3.2 for the HPLC calibration curve for tolyltriazole.

Step 2 Rearranging the equation to provide the measured concentration of tolyltriazole within the prepared 40-mL vial sample. The solution analyzed contains tolyltriazole + methanol + H_2O from the soil, making the concentration slightly diluted.

Step 3 The combined density of the methanol with H_2O, volumes, and the mass of both solution types. Data reference is from the pre-measurements found in Table H-1

- Methanol mass is determined from pre-measurements
- H_2O mass is determined form premeasurements (mass in vial * moisture content of soil)
- Methanol volume is found from the known density (TTA = 0.786) divided by its mass
- H_2O volume equals H_2O mass

Step 4 Using (step 3)*(step 4)*(Table H-1 data) / unit conversion (1L/1,000 mL)

Step 5 The mass of soil in the vial (from Table H-1)

Step 6 (step 4)/(step 5)*unit conversion of soil (1 kg/1,000 mg)

Step 7 Initial concentration of tolyltriazole in soil (mg chemical/kg soil)

Step 8 [(step 6)/ (step 7)] * 100%

The recovered tolyltriazole from interaction with the soil (without biodegradation potential) was now established for all of the possible chemical concentrations (shown above). The same procedures were followed for each of the different concentrations/residuals of tolyltriazole recovered "after" the respirometry experiments.

Measurements of Tolyltriazole Residuals After Respirometry Experiments

Table H-5
HPLC data for Tolyltriazole (25 mg/kg) Treatment of Uncontaminated Soil
(After Respirometry Experiments)

Treatment	Microcosm	Wt of 40 mL Vial (gm)	Wt Vial & Soil (gm)	Wt Vial & Soil & Methanol (gm)	Wt of Methanol in the Vial (gm)	Wt of Soil in the Vial (gm)
TTA_{25}	1	22.4438	29.3103	40.9506	11.6403	6.8665
	2	22.4796	30.0171	41.6036	11.5865	7.5375
	3	22.3229	29.5542	41.1647	11.6105	7.2313
	4	22.4637	29.7977	41.4635	11.6659	7.3339
	5	22.4028	30.3971	41.9554	11.5583	7.9943
PG_{1000}	8	22.3728	29.2256	40.8275	11.6019	6.8528
& TTA_{25}	9	22.4878	30.6921	41.9831	11.2910	8.2043
	10	22.2239	29.7320	41.4520	11.7199	7.5081
	11	22.5094	30.3521	41.9602	11.6081	7.8426
	12	22.4167	30.8103	41.5259	10.7156	8.3936

Treatment	Microcosm	Wt of Alum Cont (gm)	Wt of Alum Cont & Wet Soil (gm)	Wt of Alum Cont & Dry Soil (gm)	Wt of Wet Soil (gm)	Wt of Dry Soil (gm)	Wt of H_2O (gm)	% H_2O in Spent Soil
TTA_{25}	1	1.5590	16.3870	15.1052	14.8280	13.5462	1.2818	8.64%
	2	1.5597	18.6702	17.3878	17.1105	15.8280	1.2825	7.50%
	3	1.5587	17.0204	15.8300	15.4617	14.2713	1.1904	7.70%
	4	1.5433	19.5058	17.9913	17.9625	16.4480	1.5145	8.43%
	5	1.5404	17.6691	16.4280	16.1287	14.8876	1.2411	7.69%
PG_{1000}	8	1.5480	15.2804	14.1787	13.7324	12.6308	1.1017	8.02%
& TTA_{25}	9	1.5541	17.5317	16.0666	15.9776	14.5125	1.4651	9.17%
	10	1.5578	17.8573	16.5190	16.2995	14.9612	1.3383	8.21%
	11	1.5476	18.5609	17.0613	17.0133	15.5138	1.4995	8.81%
	12	1.5559	20.0553	18.3610	18.4994	16.8051	1.6943	9.16%

		Average HPLC Area (mAu*s)	Conc.	Density of Methanol/H_2O mix in Vial	Mass of Toly in Vial	Soil in Vial	End Conc	Initial Conc	% recovered
Treatment	Microcosms	y = 9.01x	x = y/9.01 (mg/L)	(wtH_2O+wtMeth) / ($volH_2O$+volMeth) (mg/mL)	[(conc/density)*(wt H20 + Meth)]/1000mL (mg toly)	(mg)	mg toly / kg soil	mg toly / kg soil	(End Conc/Initial Conc)*100
TTA_{25}	1	45.161	5.012	0.797	0.077	6.866	11.203	25.000	44.81%
	2	62.191	6.902	0.797	0.105	7.537	13.965	25.000	55.86%
	3	49.737	5.520	0.797	0.084	7.231	11.658	25.000	46.63%
	4	56.593	6.281	0.797	0.097	7.334	13.193	25.000	52.77%
	5	52.845	5.865	0.798	0.090	7.994	11.199	25.000	44.80%
PG_{1000}	8	39.056	4.335	0.797	0.066	6.853	9.649	25.000	38.60%
& TTA_{25}	9	42.454	4.712	0.800	0.071	8.204	8.651	25.000	34.60%
	10	44.555	4.945	0.797	0.077	7.508	10.189	25.000	40.76%
	11	50.348	5.588	0.798	0.086	7.843	10.975	25.000	43.90%
	12	56.618	6.284	0.800	0.090	8.394	10.743	25.000	42.97%

Note: All values of measurement (electronic scale or HPLC) were performed three times for each value represented in these data tables above.

Table H-6
HPLC data for Tolyltriazole (250 mg/kg) Treatment of Uncontaminated Soil (After Respirometry Experiments)

Treatment	Microcosm	Wt of 40 mL Vial (gm)	Wt Vial & Soil (gm)	Wt Vial & Soil & Methanol (gm)	Wt of Methanol in the Vial (gm)	Wt of Soil in the Vial (gm)
TTA_{250}	1	22.5350	34.3986	45.7780	11.3794	11.8636
	2	22.3719	35.5217	47.1501	11.6284	13.1498
	3	22.4041	36.2176	47.7770	11.5594	13.8135
	4	22.3553	33.1823	44.7836	11.6013	10.8270
	5	22.4222	36.2612	47.8345	11.5733	13.8390
PG_{1000}	8	22.3768	35.8517	47.4285	11.5768	13.4749
& TTA_{250}	9	22.3548	35.3941	46.8474	11.4533	13.0393
	10	22.4244	35.8969	47.3368	11.4399	13.4726
	11	22.4282	33.4309	45.1015	11.6706	11.0027
	12	22.4331	33.6359	45.1128	11.4770	11.2028

Treatment	Microcosm	Wt of Alum Cont (gm)	Wt of Alum Cont & Wet Soil (gm)	Wt of Alum Cont & Dry Soil (gm)	Wt of Wet Soil (gm)	Wt of Dry Soil (gm)	Wt of H_20 (gm)	% H_2O in Spent Soil
TTA_{250}	1	1.5644	12.0875	10.9265	10.5231	9.3621	1.1610	11.03%
	2	1.5504	13.4043	12.1118	11.8539	10.5614	1.2925	10.90%
	3	1.5521	12.9319	11.5875	11.3798	10.0354	1.3444	11.81%
	4	1.5573	15.1907	13.7298	13.6335	12.1725	1.4610	10.72%
	5	1.5588	13.5468	12.1012	11.9880	10.5424	1.4456	12.06%
PG_{1000}	8	1.5571	13.4280	12.1117	11.8709	10.5546	1.3163	11.09%
& TTA_{250}	9	1.5547	12.7645	11.5791	11.2099	10.0244	1.1855	10.58%
	10	1.5566	13.4392	11.9261	11.8826	10.3695	1.5131	12.73%
	11	1.5564	15.3807	13.8154	13.8243	12.2590	1.5653	11.32%
	12	1.5524	13.9648	12.5435	12.4124	10.9910	1.4214	11.45%

		Average HPLC Area (mAu*s)	Conc.	Density of Methanol/H_20 mix in Vial	Mass of Toly in Vial	Soil in Vial	End Conc	Intial Conc	% recovered
Treatment	Microsms	y = 9.01x	x = y/9.01 (mg/L)	(wtH_2O+wtMeth) / (volH_2O+volMeth) (mg/mL)	[(conc/density)*(wt H20 + Meth)]/1000mL (mg toly)	(mg)	mg toly / kg soil	mg toly / kg soil	(End Conc/Intial Conc)*100
TTA_{250}	1	1308.394	145.216	0.807	2.284	11.864	192.559	250.000	77.02%
	2	1581.670	175.546	0.808	2.839	13.150	215.890	250.000	86.36%
	3	1533.080	170.153	0.810	2.771	13.813	200.568	250.000	80.23%
	4	1218.012	135.184	0.804	2.145	10.827	198.075	250.000	79.23%
	5	1616.160	179.374	0.811	2.930	13.839	211.753	250.000	84.70%
PG_{1000}	8	1399.876	155.369	0.809	2.512	13.475	186.409	250.000	74.56%
& TTA_{250}	9	1432.343	158.973	0.807	2.527	13.039	193.790	250.000	77.52%
	10	1284.401	142.553	0.811	2.311	13.473	171.568	250.000	68.63%
	11	1123.405	124.684	0.805	2.000	11.003	181.739	250.000	72.70%
	12	1175.323	130.447	0.806	2.065	11.203	184.315	250.000	73.73%

Note: All values of measurement (electronic scale or HPLC) were performed three times for each value represented in these data tables above.

A summarization of Tables H-4 through H-6 is provided in Table H-7 below.

Table H-7
Percentages of Tolyltriazole Residual Recovered

Treatment	Percent of tolyltriazole residual measured through HPLC analysis					
	Before Respirometry Test (3 samples used)			After Respirometry Test (5 microcosms used)		
	Avg	Std Dev	Reference	Avg	Std Dev	Reference
TTA_{25}	99.79%	1.35%	Table H-4	48.97%	5.05%	Table H-5
TTA_{250}	90.56%	0.33%	Table H-4	81.51%	3.89%	Table H-6
TTA_{500}	95.15%	0.08%	Table H-4	No test performed	-------	-------
PG_{1000} & TTA_{25}	97.21%	1.17%	Table H-4	40.17%	3.73%	Table H-5
PG_{1000} & TTA_{250}	95.59%	0.17%	Table H-4	73.43%	3.23%	Table H-6
PG_{1000} & TTA_{500}	95.93%	0.12%	Table H-4	No test performed	-------	-------

<u>Statistical Analysis of Percent Tolyltriazole Recovered</u>

The recovered tolyltriazole after respirometry tests appears to have a lower by a difference of ~8.5% Δ ± Std Dev when in the presence of propylene glycol (Table H-8).

Table H-8
Difference in Tolyltriazole Percentage Recovered due to Propylene Glycol Presence

Treatment	Percent of tolyltriazole residual measured through HPLC analysis After Respirometry Test (5 microcosms used)	
	Avg	Std Dev
TTA_{25}	48.97%	5.05%
TTA_{250}	81.51%	3.89%
TTA_{500}	No test performed	-------
PG_{1000} & TTA_{25}	40.17%	3.73%
PG_{1000} & TTA_{250}	73.43%	3.23%
PG_{1000} & TTA_{500}	No test performed	-------

8.8% Δ ± Std

8.1% Δ ± Std

The indication was that the tolyltriazole <u>mass</u> (25 mg/kg or 250 mg/kg) degraded at a consistent amount ((8.8% + 8.1%)/2 = ~8.5%) when present with propylene glycol (1,000 mg/kg) in the soil. A two-sample t-test was used to identify if theses additional degradation percentages (8.1% and 8.8%) were similar, or if the standard deviations would dismiss the possibility.

Two sample t-test set-up

A two sample t-test, with a significance level of ($\alpha = 0.05$) was used. The null hypothesis stated below [Devore, 357-360].

H_o: The null hypothesis was that the additional degradation percentages (8.1% and 8.8%) were similar in value for the two different treatments of TTA

H_a: The additional degradation percentages were not similar in value (due to Std Dev)

$H_{o:}\ \mu_D = \Delta_0$ $\qquad \mu_D = \mu_1 - \mu_2$ $\qquad \Delta_0$ = The differences of the pairs ≈ zero

$H_{a:}\ \mu_D \neq \Delta_0$

Data:

							Average	Std Dev
1	TTA_{25}	0.4480	0.4481	0.4663	0.5277	0.5586		
	PG_{1000} & TTA_{25}	0.3460	0.3860	0.4076	0.4297	0.4390		
	Differnence =	0.1019	0.0621	0.0588	0.0980	0.1196	0.0881	0.02653
2	TTA_{250}	0.7702	0.7923	0.8023	0.8470	0.8636		
	PG_{1000} & TTA_{250}	0.6863	0.7270	0.7373	0.7456	0.7752		
	Differnence =	0.0840	0.0653	0.0650	0.1014	0.0884	0.0808	0.01565

Test statistic value:

$$t = \frac{x_{bar} - y_{bar} - \Delta_0}{S_p(1/n_1+1/n_2)^{1/2}}$$

$$S_p^2 = \frac{(n_1-1)*S_1^2 + (n_2-1)*S_2^2}{(n_1+n_2)-2} = .00004743817$$

n_1 = number of differences TTA_{25} = 5

n_2 = number of differences TTA_{250} = 5

$$t = \frac{(.0881 - .0808) - 0}{.002178(1/5+1/5)^{1/2}}$$

$t = 0.587$

t_{crit} value = $t_{\alpha/2,\,(n1+n2)-2}$ = 2.306 [Devore, 707]

Rejection region for level of test

$t \geq t_{crit}$ = Reject the null

$t \leq -t_{crit}$ = Reject the null

$.0587 \leq 2.306$

$t \leq t_{crit}$, thus we do not reject the null, and say that the additional degradation percents for the two different treatments were similar

Appendix I: Microbial Colony Population Count Results

Table I-1
Averaged Microbial Colony Population Counts (48 hr point) from Interaction with Respirometry Soil (Run-2), Chemical Concentrations of Propylene Glycol (1,000 mg/kg) and Tolyltriazole (250 mg/kg)

	Microbial Colony Populations Counted			
Dilution (mL)	Blank	TTA_{250}	PG_{1000}	PG_{1000} & TTA_{250}
0.01	>300	>300	>300	>300
0.001	52	125	161	193
0.0001	15	32	14	111
0.00001	1	1	3	6

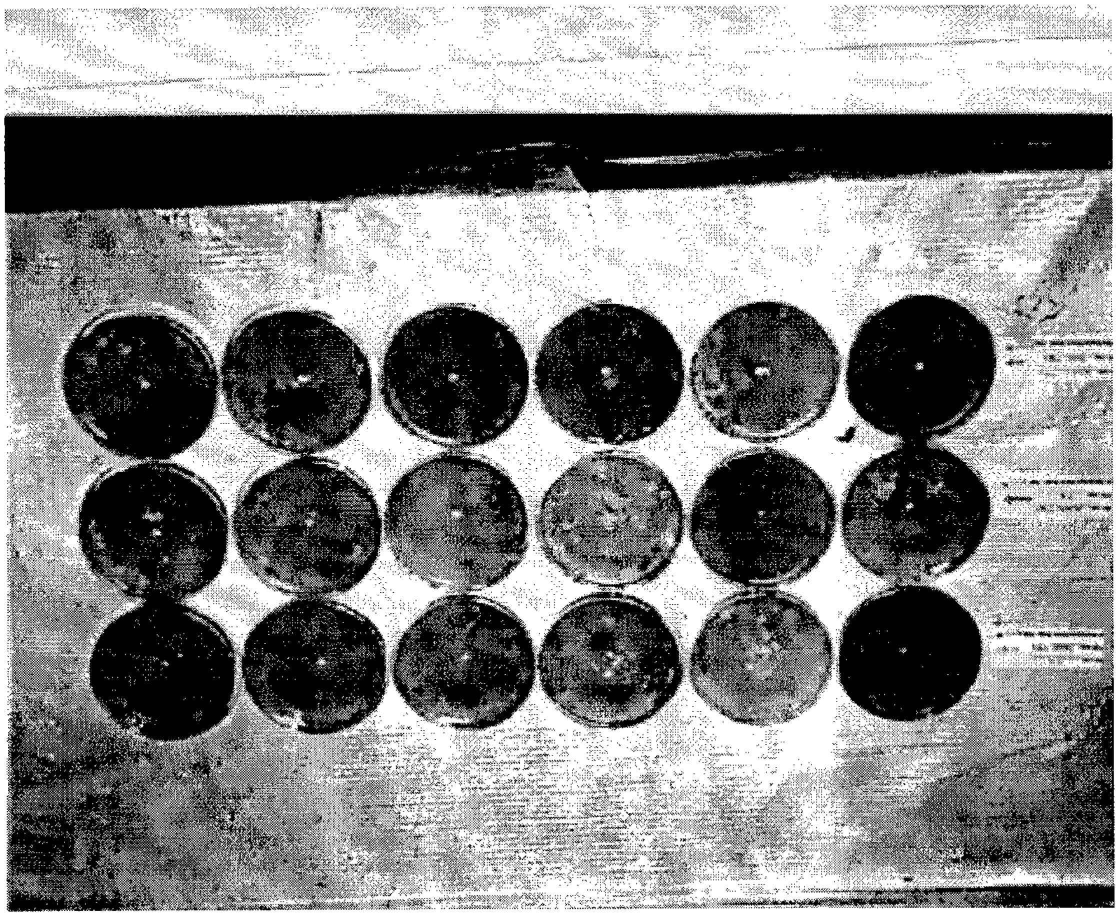
Table I-2
Averaged Microbial Colony Population Counts (48 hr point) from Interaction with Respirometry Soil (Run-3), Chemical Concentrations of Propylene Glycol (1,000 mg/kg) and Tolyltriazole (500 mg/kg)

	Microbial Colony Populations Counted			
Dilution (mL)	Blank	TTA_{500}	PG_{1000}	PG_{1000} & TTA_{500}
0.01	>300	>300	>300	>300
0.001	>300	>300	>300	>300
0.0001	110	117	201	231
0.00001	14	11	27	16

Note: Each MCPC listed (Tables I-1 and Table I-2) used three replicates, counted three times and averaged.

Appendix J: Agar Well Diffusion Test Results

Figure J-1
AWDT Visual Results (November 01, 1998)



Note: The white spots/areas represent uncolonized nutrient agar. There were <u>no</u> signs of inhibition on microbial colony growth around the well area.

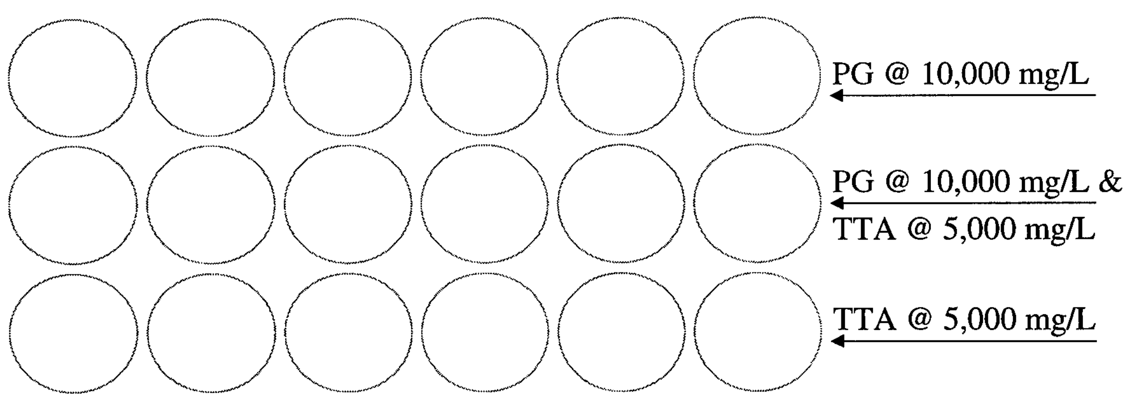
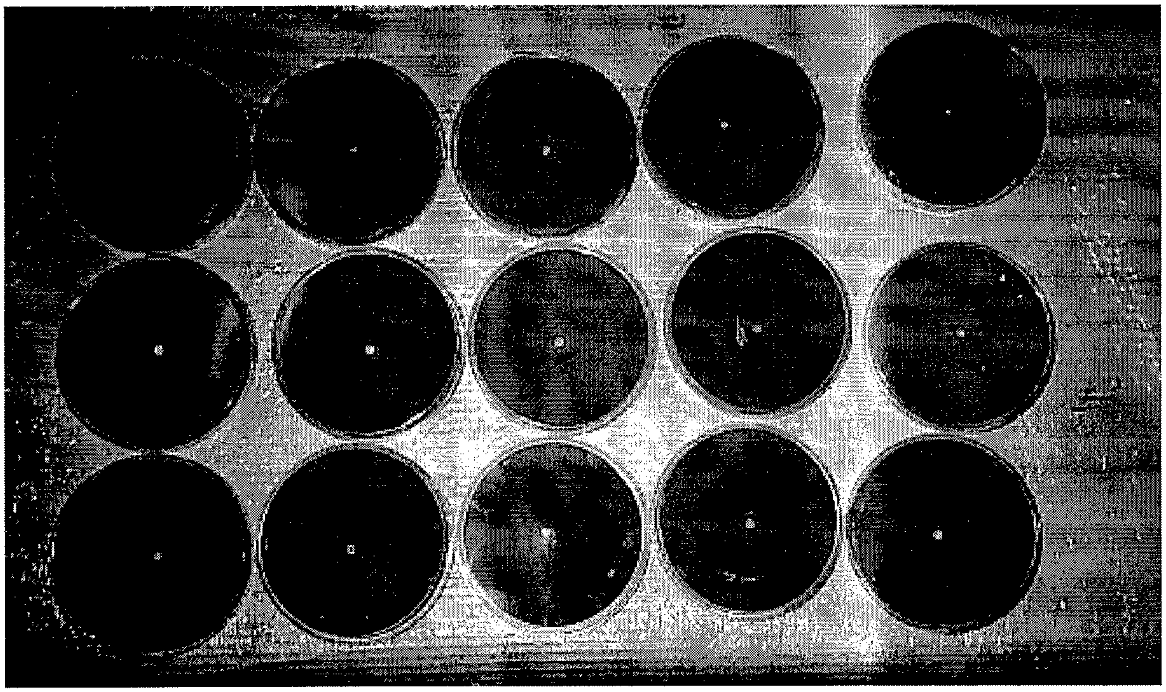
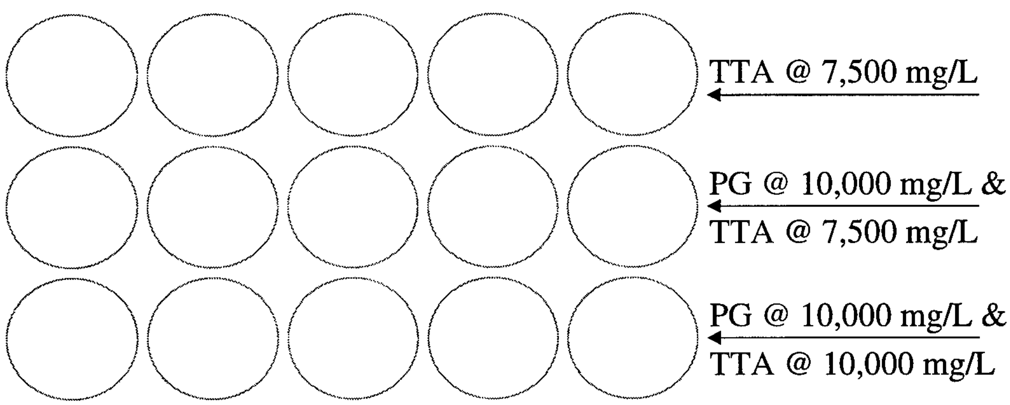


Figure J-2
AWDT Visual Results (November 29, 1998)



Note: The white spots/areas represent uncolonized nutrient agar. There were <u>no</u> signs of inhibition on microbial colony growth around the well area.



Appendix K: Theoretical Oxygen Demand Calculations

Theoretical oxygen demand (ThOD) calculations were generated from the O_2 consumption totals at the 336 hr and 468 hr points. Table K-1 summarizes the values.

Table K-1

"Actual" O_2 Consumption Totals for ThOD Calculations

Treatment	Time point O_2 totals	Avg O_2 total (uL)	Std Dev O_2 total (uL)	Blank O_2 total (uL)	V_{act} (Actual - Blank) Avg (uL)	V_{act} Std Dev (uL)
PG_{1000}	336 hr	37678	786	8808	29054	786
PG_{1000} & TTA_{25}	***468 hr***	44157	1428	10523	33633	1428
PG_{1000} & TTA_{25}	336 hr	41397	993	8992	32405	993
PG_{1000} & TTA_{250}	336 hr	49516	1898	8808	41862	1898
PG_{1000} & TTA_{500}	336 hr	52776	1716	8624	44152	1716
PG_{1000} & TTA_{750}	***468 hr***	55491	2190	10523	44967	2190
PG_{1000} & TTA_{1000}	***468 hr***	32933	2463	10523	22410	2463

Note: PG_{1000} & TTA_{25} was measured at both the 336 hr and 468 hr point to show percent biodegradation was similar (see Table K-3), using the Actual – Blank (O_2 consumption totals), thus allowing either time point to be used.

The ThOD equation for individual ADF chemical components; propylene glycol and tolyltriazole are listed in Tables 2-1 and Table 2-2, respectively. The calculation for converting milligrams (mg) to microliters (μL) of O_2 used the Ideal Gas Law. Atmospheric pressure was assumed at P = 1.00, and temperature (T) = 25°C from the respirometry runs.

Ideal Gas Law:

n = PV/RT

V = Liters (Unkown)	T = (273 + 25°C) Kelvin	n - moles O_2
L = 1×10^6 uL	R = .082058 L*atm/K*mol	MW O_2 = 32 gm/mole

Table K-2

"Total" ThOD for Available ADF Chemical Biodegradation on Uncontaminated Soil

Treatment	Mass of Chemical Available: PG (mg)	Mass of Chemical Available: TTA (mg)	ThOD for PG: $\frac{1.682 \text{ mg O2}}{\text{mg PG}}$	ThOD for PG: (uL)	ThOD for TTA: $\frac{1.564 \text{ mg O2}}{\text{mg TTA}}$	ThOD for TTA: (uL)	Total ThOD (uL)
PG_{1000}	50	0	84.10	64266	0	0	64266
PG_{1000} & TTA_{25}	50	1.25	84.10	64266	1.66	1270	65537
PG_{1000} & TTA_{250}	50	12.5	84.10	64266	16.63	12704	76971
PG_{1000} & TTA_{500}	50	25	84.10	64266	33.25	25408	89675
PG_{1000} & TTA_{750}	50	37.5	84.10	64266	49.88	38113	102379
PG_{1000} & TTA_{1000}	50	50	84.10	64266	66.50	50817	115083

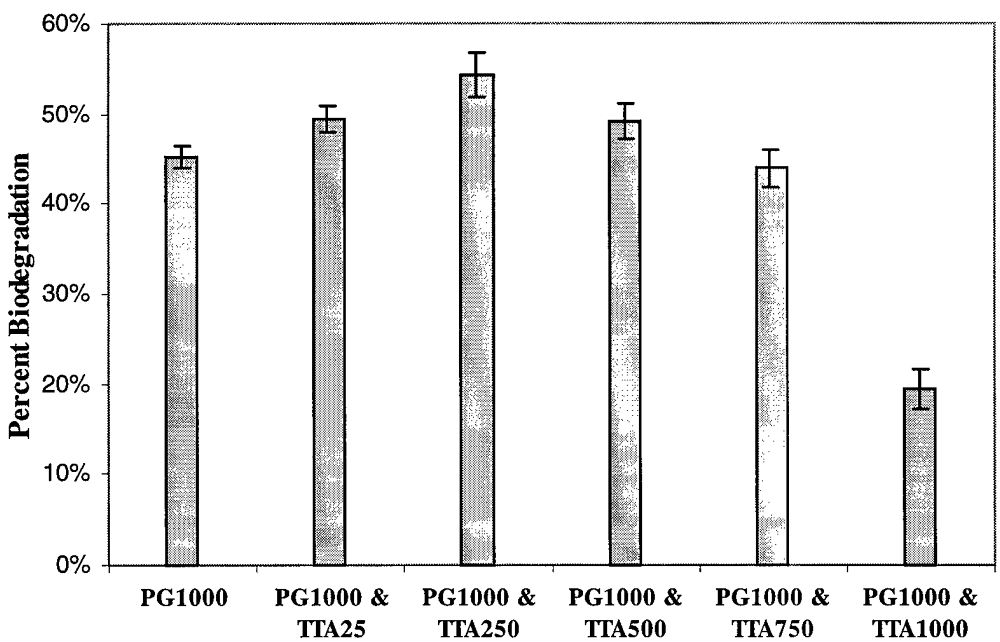
The percentage of biodegradation was generated from V_{act}/Total ThOD. Table K-3 and Figure K-1 summarize the results.

Table K-3
Percent Biodegradation from ThOD of Available ADF Chemical Components on Uncontaminated Soil

Treatment	Total ThOD (uL)	V_{act} Average (uL)	V_{act} Std Dev (uL)	% Biodegradation Average	% Biodegradation Std Dev	Time point O_2 totals
PG_{1000}	64266	29054	786	45%	1.2%	336 hr
PG_{1000} & TTA_{25}	65537	33633	1428	51%	2.2%	*468 hr*
PG_{1000} & TTA_{25}	65537	32405	993	49%	1.5%	336 hr
PG_{1000} & TTA_{250}	76971	41862	1898	54%	2.5%	336 hr
PG_{1000} & TTA_{500}	89675	44152	1716	49%	1.9%	336 hr
PG_{1000} & TTA_{750}	102379	44967	2190	44%	2.1%	*468 hr*
PG_{1000} & TTA_{1000}	115083	22410	2463	19%	2.1%	*468 hr*

similar

Figure K-1
Percent Biodegradation from ThOD of Available ADF Chemical Components on Uncontaminated Soil



Biodegradation rates in terms of mass of soil were calculated for the propylene glycol application on soil. Shown below is a sample calculation, which used Run-1, bottle/microcosm 16.

$inter := 6 \cdot hr$	Time per sampling interval, one sample per 6 hours
$number_{interval} := 56$	Number of intervals under investigation/shown below
$hours_{exp} := inter \cdot 56$	Number of hours in the experiment run = 336 hours
$hours_{exp} = 336 \circ hr$	
$v := 38996$	Microliters of oxygen consumed in treatment (336 hrs)
$v_{soil\_blank} := 8808$	Microliters of oxygen (uL) from blank soil averaged (336 hrs)
$v_{act} := v - v_{soil\_blank}$	Adjusting for background oxygen readings from blank soil (de-ionized H_2O on soil)
$V := \left(\frac{v_{act}}{1000000}\right) \cdot L$	Conversion of Microliters to Liters
$P := 1 \cdot atm$	Standard atmospheric pressure (atm)
$t := 25$	Temperature of respirometry tests (^oC)
$T := (273 + t) \cdot K$	Conversion to Kelvin (^oK)
$R := 0.082058 \cdot \frac{L \cdot atm}{K \cdot mol}$	Gas Constant (L-atm/deg K-mol)
$n := \frac{P \cdot V}{R \cdot T}$	Ideal Gas Law
$n = 0.0012 \cdot mol$	The number of moles of oxygen consumed
$soil := .050 \cdot kg$	Weight of ~60% FC soil (kg) in each microcosm
$resp_{rate} := \frac{\frac{V}{hours_{exp}}}{soil}$	$resp_{rate} = 0.02995 \circ \frac{mL}{min \cdot kg}$ **Respiration Rate (mL/min/kg) ~60% FC soil**
$ratio := 4$	Number of moles O_2 required to mineralize 1 mole $C_3H_8O_2$
$MW := 76.094 \cdot \frac{gm}{mole}$	Molecular weight of $C_3H_8O_2$ (gm/mole)
$mass_{PG} := \frac{n}{ratio} \cdot MW$	$mass_{PG} = 23.48 \circ mg$ **Mass of PG, Consumed (mg)**

$mass_{PG\_orig} := 50.0 \cdot mg$

Original mass of PG in solution added to soil

[5 mL of 10,000 PG mg/L = 50 mg PG added to 50 gm sc

$$percent_{lost} := \left(\frac{mass_{PG}}{mass_{PG\_orig}} \right) \cdot 100$$

$percent_{lost} = 46.97$

% PG Lost to Biodegradation

$spgr_{hc} := 1.0 \cdot \frac{mL}{mg}$

Specific gravity solution is considered to be 1.00 ml/mg since PG solution is mainly composed of de-ionized wat

$$degrade_{rate} := \frac{\left(\frac{mass_{PG} \cdot spgr_{hc}}{hours_{exp}} \right)}{soil}$$

$degrade_{rate} = 33.55 \cdot kg^{-1} \circ \frac{mL}{day}$

PG Biodegradation Rate, $\frac{\text{ml/day}}{\text{kg soil}}$

Appendix L: Statistical Procedures for Determining the Difference of Initial Biodegradation Rates of Uncontaminated Soil (Phase-one) compared to Acclimated Soil (Phase-two)

Overview of Statistical Test:

The statistical testing used a two-sided t-test to identify the biodegradation rates difference due to ADF components application on acclimatized microorganism/soil vs uncontaminated microorganism/soil. The statistical test used significance level of $\alpha = 0.05$.

> H_o: There was no difference between initial biodegradation rates from PG_{1000} treatment of uncontaminated compared to acclimated soil
>
> H_a: There was a difference between initial biodegradation rates from PG_{1000} treatment of uncontaminated compared to acclimated soil

Data and Calculations Performed prior to Statistical Test:

The biodegradation rates were calculated from equations used in Appendix K for the total time of 24 hrs. Run-1, Run-2, and Run-3 data was used to represent the uncontaminated soil inoculated with PG_{1000} used (15 replicates). Run-6 data was used to represent the PG_{1000} on acclimated PG_{1000} soil (5 replicates).

Note: The two soil types used blank tests (de-ionized water applied to the soil type) to measure any unusual respiration activity. The difference of the propylene glycol treatment minus the average blank (de-ionized H_2O) treatment was the O_2 total used for initial biodegradation rate. The calculations in Appendix K were used to generate the biodegradation rates per mass of soil.

Data:

Table L-1
Cumulative O_2 Consumption (336 hr point) Data for PG_{1000} Treatment on Acclimated and Uncontaminated Soil

O_2 Totals (24 hr point) (uL)						Biodegradation Rates (mL/day/kg soil)		
Run	PG_{1000} on Uncontaminated	Run-1 & 2 & 3 De-ionized H_2O on Uncontaminated	Run-6 PG_{1000} on Acclimated	Run-6 De-ionized H_2O on Acclimated	-> Calc	Run	PG_{1000} on Uncontaminated	Run-6 PG_{1000} on Acclimated
1	7525	1401	9741	1045		1	95.28	129.76
1	8190	1401	11199	1045		1	105.63	152.45
1	7183	1401	11532	1045		1	89.96	157.63
1	7468	1401	11452	1045		1	94.39	156.38
1	8111	1401	10903	1045		1	104.40	147.84
2	7583	1401				2	96.18	
2	8483	1401				2	110.19	
2	8220	1401				2	106.09	
2	8518	1401				2	110.73	
2	8316	1401				2	107.58	
3	9438	1401				3	125.05	
3	8939	1401				3	117.28	
3	9214	1401				3	121.56	
3	8870	1401				3	116.21	
3	8508	1401				3	110.58	

Wilk-Shapiro/Rankit Plot

STATISTIX® 4.0 software was used to test the distributions of each population. The test was performed to demonstrate the approximate normality of the data. Figures L-1 and Figure L-2 plots the normality for unacclimated soil and acclimated soil, respectively.

Figure L-1
Wilk-Shapiro/Rankit Plot of Initial Biodegradation Rates (24 hr point) from PG_{1000} Interaction with Uncontaminated Soil

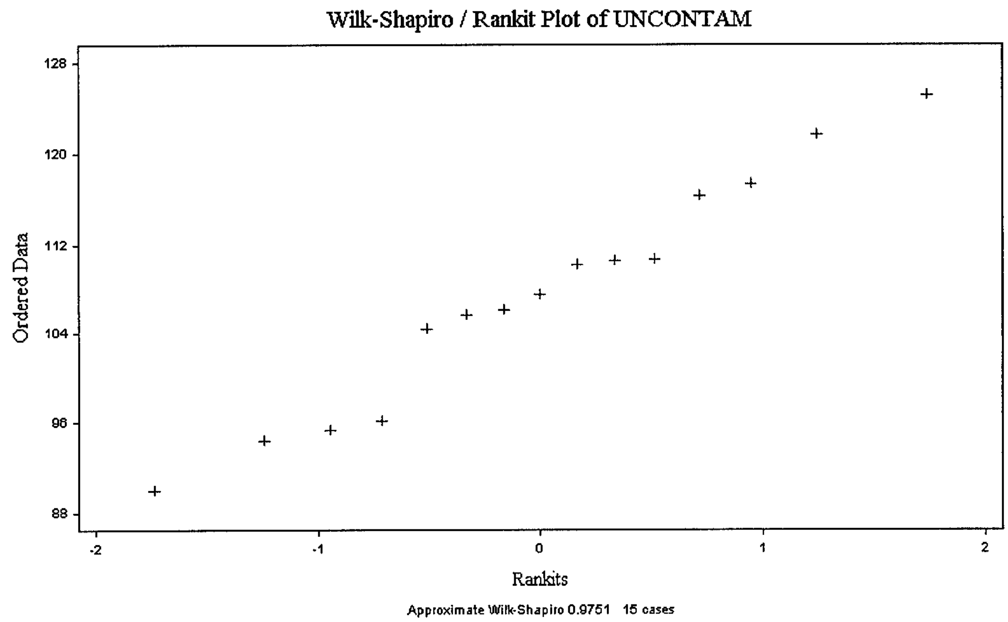
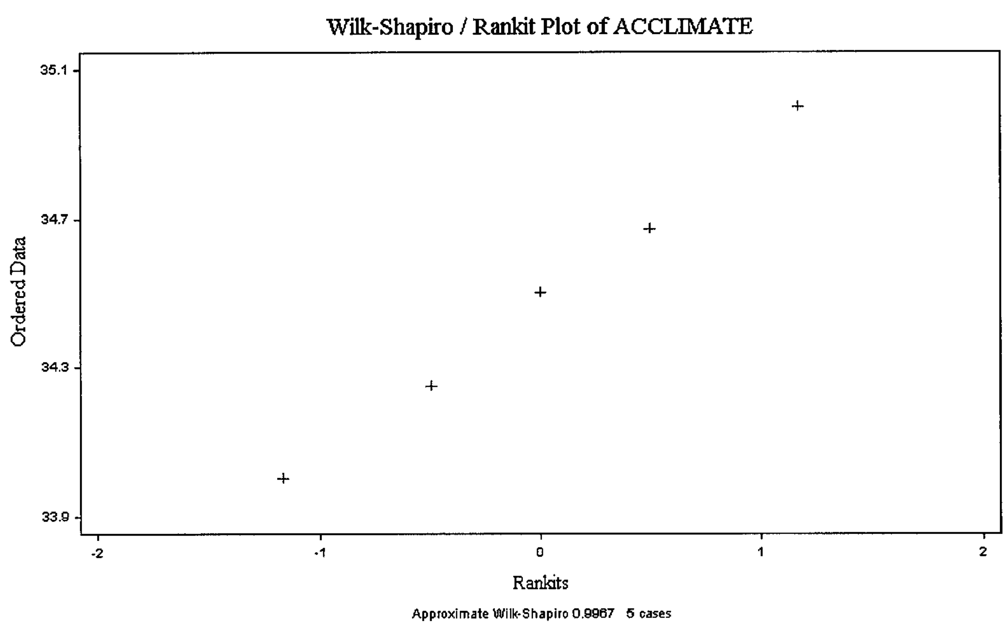


Figure L-2
Wilk-Shapiro/Rankit Plot of Initial Biodegradation Rates (24 hr point) from PG_{1000} Interaction with Acclimated Soil (PG_{1000})



Statistical Test:

The distribution of the initial biodegradation rates was approximately normal in both figures. Thus, the t-test can be performed. The averaged initial biodegradation rates for the PG_{1000} on uncontaminated soil was set as the standard mean. The mean of the initial biodegradation rates for PG_{1000} on acclimated soil (PG_{1000}) was compared to the standard mean.

Tests Statistic Value (t*):

$$t = \frac{x_{bar} - \mu_o}{(s/n^{0.5})} \qquad \text{(Devore, 291)}$$

x_{bar} = Mean of acclimated soil results (bio-rate)
μ_o = Mean of uncontaminated soil results (bio-rate)
s = Standard deviation of acclimated soil results
n = Number of replicates

T- Critical Value (t_{crit}):

T-critical (t_{crit}) was determined for a two-tailed test since the effects on biodegradation rates may be enhanced or inhibited as the alternate hypothesis. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical.

$$t_{crit} = t_{\alpha/2,\, n-1} = \pm 2.447 \quad \text{(Value from Table A.5, Devore, 707)}$$

$\alpha = 0.05$
$n = 5$ (replicates)

Rejection Region:

$-t_{crit} \le t^* \ge t_{crit}$ **If the t* value falls between the t_{crit} values, do not reject H_o**
$-2.447 \le t^* \ge 2.447$ (Devore, 318)

Summarization of Results:

Table L-2
Statistical Test of Acclimated versus Uncontaminated Soil Initial Biodegradation Rates

Averaged (Uncontaminated) u_o	Average (Acclimated) x_{bar}	Std Dev (Acclimated) s	Replicates (Acclimated) n	t-value t*	t-critical value t_{crit}	Reject H_o
107.41	148.81	11.3149565	5	27.52337	2.776	**Yes**

The null hypothesis was rejected. The conclusion was a significant <u>increase</u> in the initial biodegradation rates when PG_{1000} was applied on acclimated soil (with PG_{1000}) compared to the biodegradation rates from PG_{1000} application on uncontaminated soil.

Appendix M: Statistical Procedures for Testing the Quality/Repeatability of Data from Laboratory/Respirometry Runs

Overview of Test

The statistical analysis used a one-way ANOVA for testing the quality of laboratory procedure and the respirometry measurements through identical treatments used in the respirometry runs. The means of O_2 consumption totals, at the specific time point of 288 hrs, was used to perform the ANOVA comparisons.

There were two types of soil treatments evaluated (separately) with the statistical analysis.

1. Blank/De-ionized water on soil was performed in Run-1, Run-2, and Run-3 was used to measure the respirometers measurement quality.
   - A total of three (or more) microcosms/samples were available in each run
2. PG_{1000} application on soil was performed in Run-1 through Run-5 was used to measure the laboratory procedures/technique quality.
   - A total of three (or more) microcosm/samples were available in each run

The statistical test used a significance level of $\alpha = 0.05$

H_o: There was no difference between respirometry data sets using the same respirometer/laboratory procedures

H_a: There was a difference between (one or all) respirometry data sets using the same respirometer/laboratory procedures

$H_o = \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$

$H_a = \mu_1 \neq \mu_{i\ldots5}$

Data: Means of Cumlative O_2 (μL) from Each Experimetal Run

Table M-1
Cumulative O_2 Consumption (288 hr point) Data for De-ionized H_2O and PG_{1000} Treatments on Uncontaminated Soil

	De-ionized H_2O on Uncontaminated Soil					Average	Std Dev
Run-1	8259	8587	7947			8264	320
Run-2	7741	8526	7877			8048	420
Run-3	7681	8394	7569			7881	448

	PG_{1000} on Uncontaminated Soil					Average	Std Dev
Run-1	37907	37092	36117	37865	38773	37551	998
Run-2	43787	43530	46398	46142	44508	44873	1328
Run-3	36319	35220	35318	36193	35963	35803	505
Run-4	36455	37469	36587			36837	551
Run-5	35282	38451	38062			37265	1729

Test Statitic:

The test statistic is $F_{\alpha,v1,v2} = F_{crit}$ (Devore 709)

	De-ionized H_2O on soil	PG_{1000} on soil
Treatments number (J)	3	5
Sample size (I)	3	5
Formula degree freedom		
$v1 = I - 1$	2	4
$v2 = I(J-1)$	6	20
Info/formula above = F_{crit}	5.14	2.87

Decsion Rule:

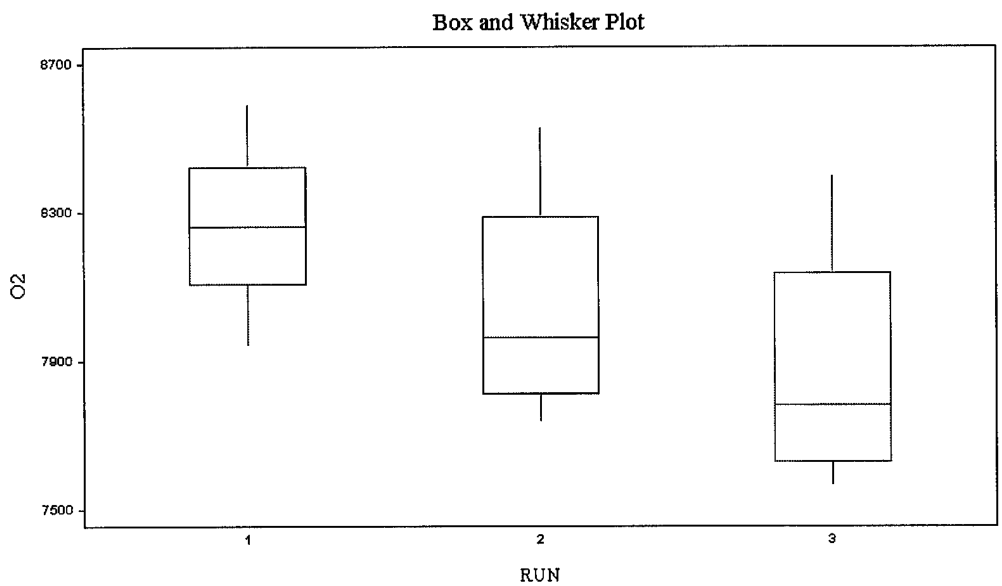
If $f^* \geq F_{\alpha,v1,v2}$ then reject the null hypothesis, else do not reject, or
If P-value $\leq \alpha$ then reject the null hypothesis, else do not reject
Formula $f^* = MSEr/MSE$

The computation of f* and relevant statistical testing data were performed with the STATISTIX® 4.1 software. The results are shown below for the two different types of soil treatments (de-ionized H_2O or PG_{1000}).

STATISTIX® Results for De-ionized H_20 on Uncontaminated soil

Outliers were checked on the data sets using a Box and Whisker plot as shown in Figure M-1.

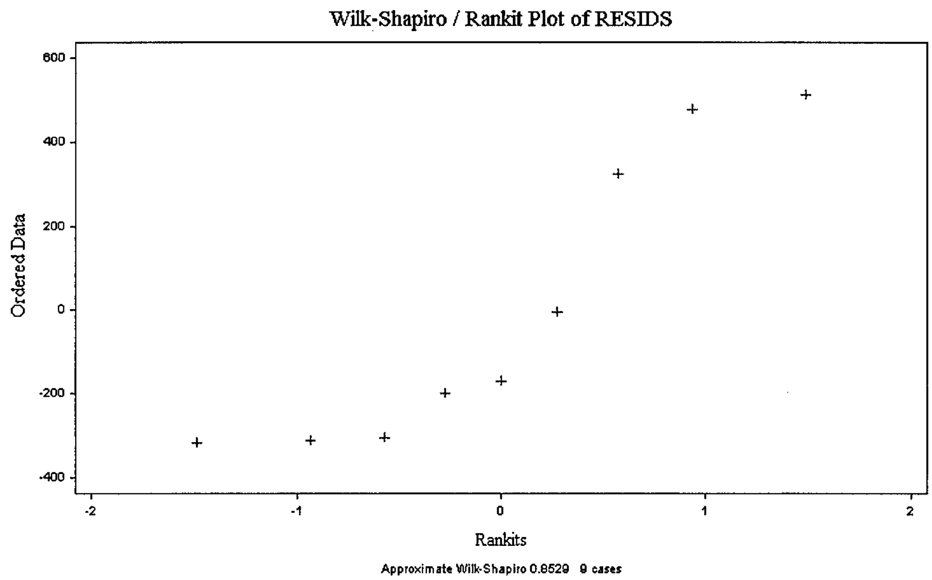
Figure M-1
Box-Whiskers Plot of O_2 totals for De-ionized H_2O on Uncontaminated Soil
(288 hr point)



Note: No outliers were apparent in any of the respirometry runs (data sets).

The one-way ANOVA produced the residuals for the three different respirometry runs. The residuals were plotted using a Wilk-Shapiro/Rankit plot as shown in Figure M-2.

Figure M-2
Wilk-Shapiro/Rankit Plot of Residuals for De-ionized H_2O on Uncontaminated Soil
(288 hr point)



The residuals show aptness (R = 0.853), thus statistical testing was continued with the one-way ANOVA results, as shown in Table M-2.

Table M-2
One-way ANOVA results for De-ionized H_2O on Uncontaminated Soil
(288 hr point)

ONE-WAY AOV FOR O2 BY RUN

SOURCE	DF	SS	MS	F	P
BETWEEN	2	221267	110633	**0.69**	**0.5359**
WITHIN	6	957329	159555		
TOTAL	8	1178596			

BARTLETT'S TEST OF	CHI-SQ	DF	P
EQUAL VARIANCES	0.19	2	0.9079

CASES INCLUDED 9 MISSING CASES 0

The decision rules were applied:

F-test:	$f^* \leq F_{crit}$	$0.690 \leq 5.14$, therefore do not reject the null
P value:	$P \geq \alpha$	$0.534 \geq 0.05$, therefore do not reject the null

A Tukey-pairwise comparison was initiated to <u>compliment</u> the one-way ANOVA results, as shown in Table M-3.

Table M-3
Tukey-pairwise of O_2 Total Means for De-ionized H_2O on Uncontaminated Soil
(288 hr point)

RUN	MEAN	HOMOGENEOUS GROUPS
1	8264	I
2	8048	I
3	7881	I

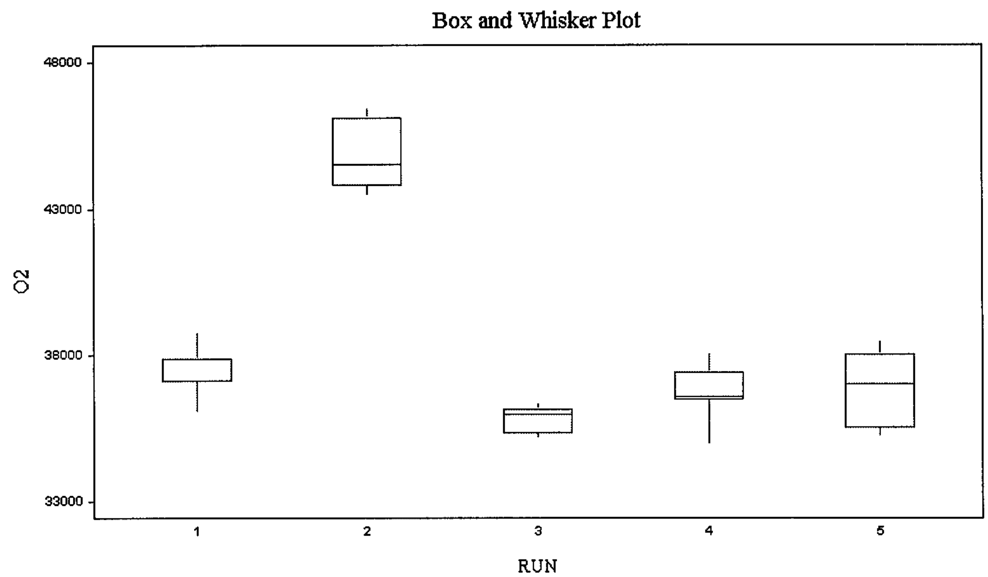
THERE ARE SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL Q VALUE 4.469 REJECTION LEVEL 0.050
STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES VARY BETWEEN COMPARISONS BECAUSE OF UNEQUAL SAMPLE SIZES.

STATISTIX® Results for PG_{1000} on Uncontaminated Soil

Outliers were checked on the data sets using a Box and Whisker plot as shown in Figure M-3.

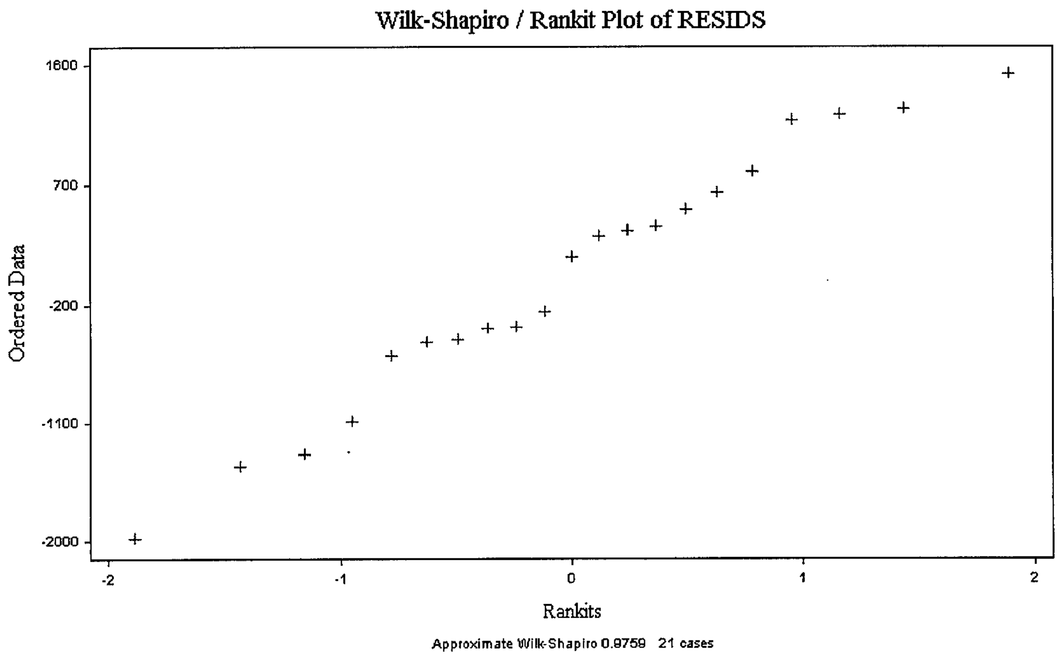
Figure M-3
Box and Whisker Plot of O_2 totals for PG_{1000} on Uncontaminated Soil
(288 hr point)



Note: No outliers were apparent in any of the respirometry runs (data sets).

The one-way ANOVA produced the residuals for the five different respirometry runs. The residuals were plotted using a Wilk-Shapiro/Rankit plot as shown in Figure M-4.

Figure M-4
Wilk-Shapiro/Rankit Plot of Residuals for PG_{1000} on Uncontaminated Soil
(288 hr point)



The residuals show aptness (R = 0.976), thus statistical testing was continued with the one-way ANOVA results, as shown in Table M-4.

Table M-4
One-way ANOVA Results for PG_{1000} on Uncontaminated Soil
(288 hr point)

ONE-WAY AOV FOR O2 BY RUN

SOURCE	DF	SS	MS	F	P
BETWEEN	4	2.557E+08	6.392E+07	**54.87**	**0.0000**
WITHIN	16	1.864E+07	1164933		
TOTAL	20	2.743E+08			

BARTLETT'S TEST OF	CHI-SQ	DF	P
EQUAL VARIANCES	5.13	4	0.2742

CASES INCLUDED 21 MISSING CASES 0

The decision rules were applied:

F-test:	$f^* \geq F_{crit}$	$54.87 \geq 2.87$, therefore reject the null
P value:	$P \leq \alpha$	$0.00 \leq 0.05$, therefore reject the null

A Tukey-pairwise comparison was initiated to determine which respirometry run means were not homogeneous, as shown in Table M-5.

Table M-5
Tukey-pairwise of O_2 Total Means for PG_{1000} on Uncontaminated Soil
(288 hr point)

RUN	MEAN	HOMOGENEOUS GROUPS	
2	44873	I	
1	37551		I
5	37265		I
4	36837		I
3	35803		I

THERE ARE 2 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE 4.469 REJECTION LEVEL 0.050
STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES VARY BETWEEN COMPARISONS BECAUSE OF UNEQUAL SAMPLE SIZES.

The results in Table M-2 and Table M-3 showed consistency from the respirometer, since the background soil treated with de-ionized water had mean O_2 consumption total that were consistent. The results of Table M-4 and Table M-5 revealed a significant difference in Run-2 compared to the other respirometry runs. This required Run-2 to be re-accomplishment.

New Data: Means of Cumlative O_2 (µL) from Each Experimetal Run

Run-2 was re-accomplished and then replaced the old Run-2 data. The new data set is listed in Table M-6.

Table M-6
Cumulative O_2 Consumption Totals (288 hr point)
(Run-2, re-accomplished and included)

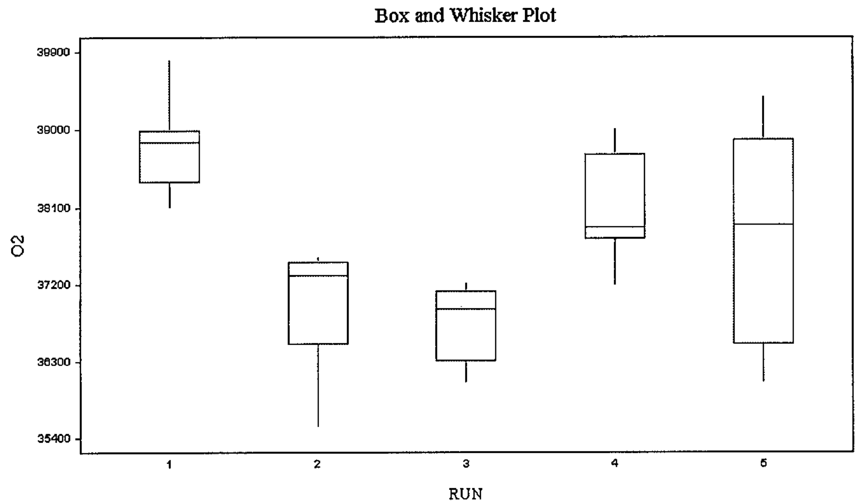
	PG_{1000} on Uncontaminated Soil					Average	Std Dev
Run-1	37907	37092	36117	37865	38773	37551	998
New Run-2	34871	36791	35905	36823	36634	36205	834
Run-3	36319	35220	35318	36193	35963	35803	505
Run-4	36455	37469	36587			36837	551
Run-5	35282	38451	38062			37265	1729

STATISTIX® Results for PG_{1000} on Uncontaminated Soil (288 hr point)

Outliers were checked on the data sets using a Box and Whisker plot as shown in Figure M-5.

Figure M-5

Box and Whisker Plot of O_2 totals for PG_{1000} on Uncontaminated Soil (288 hr point)

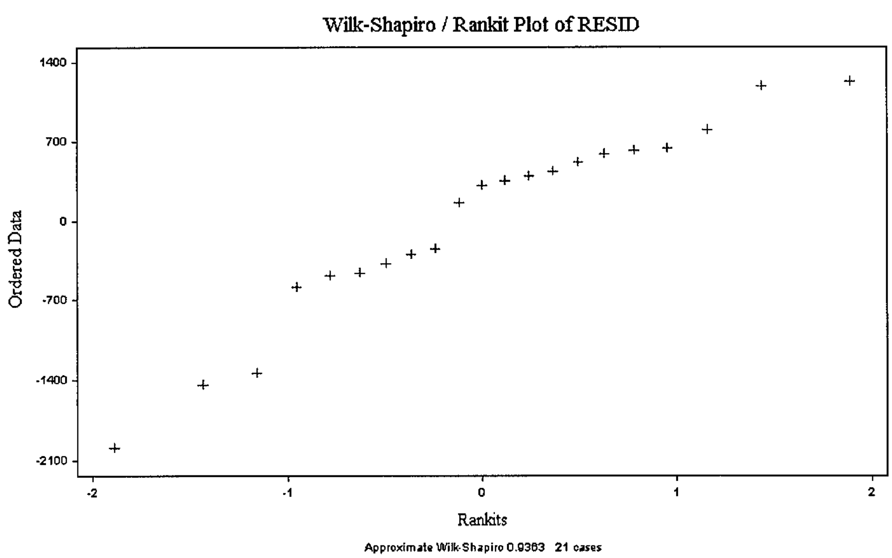


Note: No outliers were apparent in any of the respirometry runs (data sets).

The one-way ANOVA produced the residuals for the five different respirometry runs. The residuals were plotted using a Wilk-Shapiro/Rankit plot as shown in Figure M-6.

Figure M-6

Wilk-Shapiro/Rankit Plot of Residuals for PG_{1000} on Uncontaminated Soil (288 hr point)



The residuals show aptness (R = 0.936), thus statistical testing was continued with the one-way ANOVA results, as shown in Table M-7.

Table M-7
One-way ANOVA Results for PG_{1000} on Uncontaminated Soil
(288 hr point)

ONE-WAY AOV FOR O2 BY RUN

SOURCE	DF	SS	MS	F	P
BETWEEN	4	9868719	2467180	**2.75**	**0.0649**
WITHIN	16	1.437E+07	897849		
TOTAL	20	2.423E+07			

BARTLETT'S TEST OF EQUAL VARIANCES	CHI-SQ	DF	P
	4.74	4	0.3147

CASES INCLUDED 21 MISSING CASES 0

The decision rules were applied:

F-test:	$f^* \geq F_{crit}$	$2.75 \leq 2.87$, therefore do not reject the null
P value:	$P \leq \alpha$	$.065 \geq 0.05$, therefore do not reject the null

A Tukey-pairwise comparison was produced (Table M-8) to confirm the one-way ANOVA results.

Table M-8
Tukey-pairwise of O_2 Total Means for PG_{1000} on Uncontaminated Soil
O_2 Totals from Respirometry Runs (288 hr point)
(Run-2, re-accomplished and included)

RUN	MEAN	HOMOGENEOUS GROUPS
1	37551	I
5	37265	I
4	36837	I
2	36205	I
3	35803	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

The results of Table M-8 revealed that the respirometry runs were now homogenous.

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Vita

Captain Baron W. Burke was born on June 30, 1969 in Seattle, Washington. He graduated from Seattle Lutheran High School in 1987, and attended South Seattle Community College where he earned an Associate of Arts degree in December 1990. In concurrence he attended the University of Washington and graduated with a Bachelor of Science degree in Civil Engineering in June 1993. He was commissioned as a 2nd Lieutenant in the U.S. Air Force on June 11, 1993. He entered active duty service on December 1, 1993 working as a base civil engineering officer at the 92nd Civil Engineering Squadron, Fairchild A.F.B., Spokane, Washington. From May 1995 to August 1995, he was sent on temporary duty to Soto Cano Air Base, Honduras as a base civil engineering officer. From July 1996 to September 1996, he was TDY at Dhahran A.B., Saudi Arabia, as a base civil engineering and programming officer. He was subsequently selected to study for his Masters of Science in Engineering and Environmental Management at the Air Force Institute of Technology from July 1997 to March 1999. Upon completion of the AFIT program, Captain Burke will be assigned to 366th Civil Engineering Squadron at Mountain Home A.F.B., Idaho.

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BARON W. BURKE, Capt, USAF

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Air Force Institute of Technology
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Wright Patterson AFB, OH 45433-7765

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13. ABSTRACT *(Maximum 200 words)*

Aircraft de-icing fluids (ADFs) are used worldwide to ensure safe aircraft operations. This research effort was conducted to analyze the biodegradation effects of two chemical components of ADFs, propylene glycol (PG) and tolyltriazole (TTA), in a high-clay soil. The research used four test methods; automated respirometry, high performance liquid chromatography (HPLC), microbial colony population counts (MCPC), and agar well diffusion tests (AWDT). The research was partitioned into two phases of investigation. Phase-one analyzed individual and combined ADF chemical components on uncontaminated soil. The presence of TTA, from 25 - 1,000 mg/kg, reduced the maximum respiration rate of 1,000 mg/kg PG alone; however, cumulative respiration over the two-week study period was proportionality higher for TTA (25 - 500 mg/kg). Rates and respiration totals for soil exposed to TTA (25 - 750 mg/kg) alone, were not significantly different from background soil; however, rate and respiration totals for PG (1,000 mg/kg) alone were significantly higher. The HPLC percentage of recovered TTA, with or without PG presence, indicated a loss (biodegradation and/or absorption) of TTA within the soil. Kellner (1999) conducted HPLC for absorption/desorption of TTA on the same (high-clay) soil. MCPC and AWDT indicated no measurable toxic effects to microbial populations/health occurred from ADF chemical components. Phase-two research conducted reapplication of ADF chemicals on acclimated soils from phase-one. Initial respiration rates from application of 1,000 mg/kg PG on acclimated soil (PG 1,000 mg/kg) compared to 1,000 mg/kg PG on uncontaminated soil. The acclimated soil produced a significantly larger initial rate of respiration.

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Biodegradation, High Performance Liquid Chromatography, Respirometry, Propylene Glycol, Soil, Tolyltriazole, Microbial Colony Population Count, Agar Well Diffusion Test

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